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Hyperostosis frontalis interna in males: Morphological changes of bones at macro and micro levels

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Hiperostoza frontalne kosti kod muškaraca: makro i mikro morfološke promene kostiju

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Hyperostosis frontalis interna in males: Morphological changes of bones at macro and micro levels

Abstract

Introduction: Hyperostosis frontalis interna (HFI) is an idiopathic condition manifested by bone formation involving the endocranial surface of the frontal bone. A low androgen level seems to be an important correlating factor for the origin of the condition in males, while in females the correlation seems to be more complex. If hormonal imbalance was to be considered a probable cause of HFI occurrence, it would be expected that changes in hormonal levels would have some systemic effect, especially affecting the skeletal system.

Aim: To compare macroscopic and microarchitectural morphology between male and female skulls with different types of HFI, whether frontal bone formation accompanies changes in bone structure in a systemic manner and could HFI be used as an additional criterion for sex and age estimation of the skeletal remains.

Materials and methods: The first part was an observational, cross-sectional autopsy study where the sample was taken from human donor cadavers and divided into four groups: males with and without HFI and females with and without HFI. We analyzed age distribution, macroscopic appearance of HFI and morphological features (thickness of the frontal, temporal and occipital bones and longitudinal and frontal diameters of the skulls) of the male skulls with HFI and compared the results with female skulls with this condition. The second study was carried out on selected, age-matched subjects. The specimens of the frontal and femoral bones were collected and scanned using microcomputed tomography and dual energy x-ray absorptiometry (DXA). Parameters of hip structure analysis were calculated from data derived from DXA scans.

Results: Males are younger at the time of HFI occurrence and show the same risk of having this condition as females less than 55 years of age. They most commonly have milder forms of HFI. Females have almost four times greater chances of developing the most severe HFI type. Bone formation is most pronounced on the frontal bone;

however, other skull bones are affected as well. In males, HFI does not affect cranial vault size and in females the longitudinal diameter of the skull is slightly decreased. The microarchitectural structure of the frontal bone with HFI is the same in both sexes. In males, femoral bone mineral content and density tend to increase with the occurrence of a more severe form of HFI. These parameters didn't differ among females without HFI and those with moderate and severe HFI types. Males and females with HFI could be more prone to femoral neck fracture due to axial compressive forces, compared to their age-matched controls. Compared to females, males with HFI show better resistance to torsion forces in the femoral neck; otherwise, there were no differences between subjects with HFI regarding hip structure analysis. Males have denser trabecular bone with trabeculae that are closer together, while females have denser cortical bone which is less porous. However, there are no femoral microarchitectural changes in subjects with HFI, regardless of sex, either in the trabecular or the cortical compartment. HFI could be used in forensic pathology as a supplementary to other established methods for estimating sex and age of unidentified human skeletal remains.

Conclusions: Apart from the skull, the same etiological factor behind HFI most probably induces changes at the level of bone microarchitecture at a remote skeletal site (femoral bone) in both sexes, regarding both quantitative parameters and spatial microarchitectural organization. HFI could be a systemic phenomenon that affects both males and females in a similar manner.

Key words: *hyperostosis; frontal bone; skull; autopsy; male; estrogen; androgen; femur; micro-architecture; anthropology*

Scientific field: Medicine

Specific scientific field: Skeletal biology

Hiperostoza frontalne kosti kod muškaraca: makro i mikro morfološke promene kostiju

Sažetak

Uvod: Hiperostoza frontalne kosti (lat. *hyperostosis frontalis interna* – HFI) predstavlja zadebljanje unutrašnje ploče frontalne kosti čiji je uzrok nepoznat. Izgleda da je kod muškaraca nizak nivo androgena važan faktor u nastanku HFI, dok je kod žena hormonski uticaj složeniji. Ako HFI zaista nastaje usled hormonske neravnoteže, očekivano je da promene koncentracija hormona imaju uticaja i na drugim mestima u organizmu, što bi se posebno odrazilo na koštani sistem.

Cilj: Uporediti makroskopsku morfologiju i mikroarhitekturu muških i ženskih lobanja sa različitim stadijumima HFI, utvrditi da li zadebljanje čeone kosti prati sistemska promena strukture kostiju i da li se HFI može koristiti kao dodatni kriterijum za procenu pola i starosti skeletnih ostataka.

Materijal i metode: Prvi deo istraživanja je opservaciona, autopsijska studija preseka spovedena na kadaveričnom materijalu koji je podeljen u četiri grupe: muškaraci sa i bez HFI i žene sa i bez HFI. Analizirali smo starosnu distribuciju, makroskopske i morfološke karakteristike lobanja (debljinu frontalne, temporalne i okcipitalne kosti, uzdužni i poprečni dijametar) muškaraca sa HFI i upoređivali ih sa lobanjama žena sa HFI. Drugi deo istraživanja sproveden je na odbranim slučajevima iz prethodnog uzorka, koji su upareni po starosti. Uzimani su uzorci frontalne i butne kosti i analizirani korišćenjem mikrokompjuterizovane tomografije i dvoenergetske rendgenske apsorcimetrije (DXA). Parametri strukturne analize izračunati su iz podataka dobijenih DXA analizom.

Rezultati: U momentu nastanka HFI, muškarci sa mlađi od žena, a njihov rizik da imaju ovo stanje je isti kao i kod žena mlađih od 55 godina. Muškarci najčešće imaju blaže stadijume HFI. Žene imaju skoro četiri puta veću šansu da ispolje najteži stadijum HFI. Koštano zadebljanje je najizraženije na frontalnoj kosti kod svih osoba sa HFI, međutim, ostale kosti lobanje su takođe zahvaćene procesom. Kod muškaraca, HFI ne utiče na zapreminu lobanjske duplje, dok je kod žena sa HFI uzdužni dijametar lobanje lako smanjen. Mikroarhitektura frontalne kosti sa HFI je ista u oba pola.

Ukupna količina mineralne materije i koštana gustina butne kosti koreliraju sa težim stadijumima HFI kod muškaraca, što kod žena nije bio slučaj. Osobe sa HFI oba pola su podložnije prelomu vrata butne kosti nastalom dejstvom kompresivnih sila, u poređenju sa kontrolama uparenim po starosti. Parametri strukturne analize butne kosti se ne razlikuju između muškaraca i žena sa HFI, osim u predelu vrata butne kosti gde muškarci pokazuju bolju otpornost na sile savijanja. Muškarci sa HFI imaju veću gustinu trabekularne kosti i trabekule su međusobno bliže. Žene sa HFI imaju veću gustinu kortikalne kosti, koja je zbog toga manje porozna. Međutim, upoređivanjem parametara mikroarhitekture butne kosti između muškaraca i žena sa HFI nisu dobijene razlike, ni u trabekularnoj ni u kortikalnoj kosti. U sudskoj medicini, HFI se može koristiti kao dodatni kriterijum za procenu pola i starosti skletenih ostataka, uz korišćenje ostalih metoda forenzičke antropologije.

Zaključak: Osim što utiče na kosti lobanje, isti etiološki faktor koji dovodi do nastanka HFI najverovatnije deluje i na udaljenim mestima (na butnu kost) i to kod oba pola, što se ispoljava kroz promene kvanitativnih i kvalitativnih parametara mikroarhitekture butne kosti. HFI bi mogao biti sistemski fenomen koji se ispoljava kod oba pola na sličan način.

Ključne reči: *hiperostoza; frontalna kost; lobanja; obdukcija; muškarac; estrogen; androgen; butna kost; mikroarhitektura; antropologija*

Naučna oblast: Medicina

Uža naučna oblast: Biologija skeleta

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1 Introduction

1.1 Hyperostosis frontalis interna

While preforming an autopsy of an obese, elderly woman in 1719 pathologist Giovanni Batista Morgagni noticed and described "bone accretion localized on the inner table of the frontal bone" [1]. More than 300 years later, the thickenings of the inner surface of the skull are still receiving attention in pathologic, anatomical and radiological literature.

After his study on skull radiographs, Moore introduced the classification of calvarial hyperostosis in 1936, based primarily on the location of bone formation on the endocranial plate. It included: (1) *nebula frontalis* – a smooth, uniform thickening of the frontal bone, (2) *hyperostosis frontalis interna (HFI)* – irregular thickening of the frontal bone, (3) *hyperostosis frontoparietalis* – extension of the previous to the parietal bones and (4) *hyperostosis calvaria diffusa (HCD)* – diffuse thickening of all the bones of the calvarium [2–4]. At the time, Moore considered HFI to be a precursor to HCD [5]. Perou [6] offered the term *hyperostosis cranii interna* to describe all cases of cranial endostosis, regardless of their endocranial location. Finally, 20 years ago Hershkovitz et al. separated HFI from all other cranial hyperostoses and defined it as "a disorder of the endocranial plate that remodels into a more cancellous phenotype" [7]. Given that this research group conducted the largest sample size study on this subject (3 797 skulls from three different time periods, varied geographic locations and ethnicities), which was followed by a very extensive review and proposition of HFI pathogenesis, this definition, albeit broad and, to some extent, imprecise, is frequently cited in literature. Since the etiology of this condition remains unknown, in our opinion the most suitable definition of HFI could be "an idiopathic condition manifested by the bone formation involving the endocranial surface of the frontal bone"[8].

1.2 Diagnosis and differential diagnosis of HFI

HFI could be diagnosed during radiological imaging of the skull or at the autopsy. On a plain X-ray film, HFI is characterized by midline sparing of the frontal bone resulting in characteristic "butterfly-like" density [9]. It is most commonly seen by

radiologists as an incidental finding and should not to be mistaken for other kinds of skull pathology.

At the autopsy, upon opening the skull using the standard technique, HFI could be observed on the skull-cap and in the most severe cases on the skull base (at the orbital part of the frontal bone). It may vary in shape and size, ranging from small single or multiple elevations to extensive, continuous bony overgrowth which affects different percentage of the endocranial surface of the frontal bone. The margins of individual foci are sometimes well-defined and other times difficult to distinguish. In milder cases, frontal bone changes are limited to the anterior, parasagittal aspects. Intermediate stages are characterized by the loss of margination, increased osseous thickening and the involvement of a greater proportion of the frontal bone. The most advanced cases occupy the entire frontal bone, usually in the form of cauliflower-shaped lobulations with sharp posterior margins. Lesions are usually restricted to the area between the superior sagittal sinus and the groove of the middle meningeal artery, which are, as a rule, free of HFI. Not even the most severe cases of HFI cross the suture lines. Typically, the pattern of bone deposition is bilateral and the degree of symmetry increases with the extent of involvement. The endosteal component of the dura associated with HFI is usually firmly attached to the bone and very thin, sometimes with fibers running under the bony ridges [7].

The differential diagnosis of HFI includes various sources of cranial hypertrophy which can affect only the skull or be systemic in nature [5–7,9]. Chronic subdural hematomas, meningiomas and osteomas can induce localized calcification. Pregnancy osteophytes can occur in the cranial vault, but are typically more frequent on the ectocranial surface and tend to prefer areas of venous sinuses. Paget's disease, acromegaly and fibrous dysplasia are systemic disorders which can affect any of the skull bones, usually the outer skull table or spongiosa. Leontiasis ossea (leontiasis or lion's face) is a rare medical condition, characterized by an overgrowth of the facial and cranial bones, secondary to advanced leprosy, hyperparathyroidism and renal osteodystrophy. However, the typical features of HFI (unaffected midline, tendency towards bilateral symmetry and clear boundaries along the middle meningeal artery) are sufficient to distinguish these conditions [7].

1.3 HFI classifications

According to severity, HFI can be classified using radiological (indirect) or macroscopic (direct) methods. Littlejohn proposed the radiological criteria for the classification of diffuse idiopathic skeletal hyperostosis [10] which Barber et al. [11] suggested as appropriate for describing HFI in plain radiographs: 0 – no new bone formation; 1 – early endosteal new bone on the inner table; 2 – more advanced endosteal bone covered in bosses (protuberances); 3 – severe change with much irregularity and increased thickness. Hershkovitz et al. [7] reclassified HFI into four types (A–D) based on the location on the frontal bone, the extent of involvement, appearance, shape, border type and involvement of other bones. This classification is now widely used in medical literature and described in detail in the Material and Methods section of this research. Some authors have even extended this classification by adding "type E" which corresponds to severe HFI with soft tissue expansion (namely, hyperostotic finding at the falx cerebri) [12].

When using computed tomography (CT) images to detect HFI, May et al. suggested using a modified version of Herskovits's categorization method, i.e. reducing the number of categories to three: the "No HFI" category merges with type A, while types B–D remain as described [13]. They suggested that this rating method would reduce the risk of "overrating" type A due to amplification of HFI as the result of volume rendering in CT imaging. Finally, Bracanović at al. showed that the macroscopic grades of HFI could not be distinguished at the level of bone microarchitecture using micro-CT, and suggested that only two different types of HFI (moderate, or previous types A–C, and severe, or previous type D) should be considered [14].

1.4 The prevalence of HFI

The prevalence or the proportion of a particular population found to be affected by a condition is very hard to estimate in the case of HFI. Different available methods of observation and lesion classification have led to substantial variation and inconsistency in the prevalence rates of HFI recorded to date, even within the same study populations [8]. Since its clinical significance is still subject to discussions and

the condition lacks specific symptoms, HFI is usually an incidental finding on radiographic imaging and its prevalence in the general population can only be assumed. It should be kept in mind that these lesions are frequently found in the antero-medial portion of the frontal bone and are often difficult to detect on radiographs, because of the superimposition of cranial structures in the frontal projection [15], therefore leading to underestimation. Some authors believe that, when autopsy material is taken into account, younger individuals might be underrepresented in cadaveric study groups, which is why the prevalence rates of HFI among this group in modern populations can only be reliably assessed using CT scans of living individuals [8]. Conversely, in archeological studies, older-aged individuals are consistently underrepresented or misidentified as the result of consistent underestimation of their age [16].

However, Moore reported that HFI occurs in 5-12% of the general population [5]. Regarding sex distribution, the reported rates are around 20% for females and 5% for males [7,8,17,18]. Some authors even suggest that female to male ratio is 9:1 [19], while others disagree and explain that males tend to have milder forms of HFI which are more commonly unrecognized, especially when using plain radiographs [7]. Most reports of HFI in archeological remains are isolated cases [15,20,21], with a few exceptions: Lazer [22] reported observing HFI in 43 out of 360 skulls (11.9%) from Pompeii and Mulher [23] observed it in 12 out of 37 adults (32.4%) in archeological site Pueblo Bonito.

The following general conclusions about the prevalence of HFI could with certainty be drawn from all previous research: (1) this condition is most frequently observed in postmenopausal females [7,8,18]; (2) HFI is a contemporary phenomenon, not commonly present in archeological samples [7,8,15,22,23]; (3) during the last century HFI prevalence seems to be showing continuous increase which has gained momentum over the last two decades [7,13].

1.5 Histopathology of HFI

There are a few studies which included the use of standard and special staining along with light and polarizing microscopy to analyze HFI histopathology [7,12,24–26]. We combined several of those descriptions. The outer table consists of normal dense lamellar bone with regular Haversian structure and no evidence of bone remodeling or

resorption. In the internal part of spongiosa the trabeculae are grown together which many authors describe as the *sclerotic zone*. The marrow cavities along with these trabeculae have an irregular shape and size pattern. If bony nodule is present, it arises from the surface of the inner table, and the histological section shows the large cavities (probably vascular sinuses), forming a "ballooning area" (some authors described this as the *pneumatisation of the diploë* [25]). These sinuses are separated from each other by bony walls made of disorganized lamellar bone. The nature of these bony formations is characterized by their morphological structures as a very slowly growing process. A thin, concave shell of organized lamellar bone covers the inner table in places where there are no bony nodules. Hershkovitz defined all of the above as "five distinctive osseous layers" and underlined that they represent sequential stages of a single process [7].

1.6 Pathophysiology of HFI

1.6.1 *The involvement of the frontal bone*

The skull is formed by 23 bones and one of those is the unpaired frontal bone (*os frontale*). It consists of the vertical portion (squamous part) that forms the forehead and the horizontal portion that forms the roofs of orbits and the nasal cavity (orbital and nasal part). The frontal bone is a flat bone, composed of two thin layers of compact bone (outer and inner tables) with a variable amount of spongy, cancellous bone between them (spongiosa). The outer table is thick and tough, while the inner table is thin, dense and brittle. The inner table of the frontal bone is covered with dura mater, which is the source of blood supply: it is vascularized by anterior meningeal branches of the anterior and posterior ethmoidal arteries and internal carotid, and a branch from the middle meningeal artery. The veins returning the blood from the cranial dura mater anastomose with the diploic veins and end in various sinuses [27].

During embryological and fetal development, the skull is formed from cranial skeletogenic mesenchyme derived from two distinct embryonic sources: mesoderm and neural crest [28]. The neural crest cells present a population of pluripotent cells that migrate from the margins of the neural tube. The frontal bone and metopic suture are composed exclusively from neural crest tissue, while the parietal bone and sagittal

suture are derivatives of paraxial mesoderm. The dura mater underlying both tissues originates from the neural crest. It participates in the regulation of growth, patency, and cellular phenotypes of the cranial sutures [29].

Like all flat bones, the frontal bone is ossified by the mechanisms of intramembranous ossification [30]. This process starts in two primary centers, one for either half of the bone, localized above the supraorbital margins. From both of these centers, ossification extends upward, to form the corresponding half of the squama, and backward, to form the orbital plate [27]. At the cellular level, osteoblasts accumulate at the periphery of the ossification center and continue to secrete osteoid toward the center of the nodule. As the process continues, osteoid undergoes mineralization and trapped osteoblasts become osteocytes [30].

The presentation of HFI in the frontal bone is still open to discussions. Since the process almost always begins in the middle third of the frontal squama, Morel suggests that the point of origin corresponds with the original centers of ossification of the bone [7]. These centers are bilaterally active during adulthood [7]. For some reason, the frontal bone is considered a "favored hormone target" [7] and Calame suggested that estrogen stimulus may reactivate the primary centers of ossification of the frontal bone, causing abnormal bone growth [31]. Hershkovitz agrees and notes that the bilateral occurrence of HFI, as well as the fact that hyperostosis is limited to areas associated with the ossification centers and not to the midline (metopic suture) and bregmatic area (anterior fontanel), further support the primary ossification center involvement [7]. More recent research has demonstrated that a higher expression of fibroblast growth factor ligands and 1, 2 and 3 receptors in the frontal bone leads to a specific increased capacity of the frontal bone to regenerate, whereby dura mater and pericranium cells contribute and migrate to calvarial re-growth [32]. There is also a theory that the frontal bone may be involved because of its special vascularization. HFI is frequently observed in proximity to a depression which may contain vascular openings [6] and the grooves between the bony ridges of HFI are occupied by veins exiting the spongiosa [7].

1.6.2 *Models of HFI pathogenesis*

Three models are proposed to explain the pathophysiology of HFI. The main difference between these models is the uncertainty about whether HFI represents bony

growth or bony *deposition* and where this process actually starts. In all models, there is a consensus that the outer table of the frontal bone is not affected.

The "American Model", proposed by Moore [5], describes HFI as a process that triggers proliferation of spongy bone and that increase in diploic volume is pushing the inner table towards endocranial structures. Thevoz [33] suggested the "European Model" where he defined HFI as a process which transpires exclusively in the dura and is triggered by the enlargement of the intradural vasculature. The most recent is the "Global Model" proposed by Hershkovitz et al. [7] (called "global" due to varied nationalities of the contributors). According to this model, HFI begins when osteogenic cells cause a disorganized diploization process in the inner table. These changes trigger the superimposition of the newly formed lamellae on the inner table by the periosteum. The early compact hyperostosis is mainly composed of new lamellar bony layers deposited by the dura. Then, numerous blood vessels penetrate the lamellar bone from the dura, inducing bone proliferation. Over time, the original inner table becomes sclerotic and the newly formed bone undergoes dramatic reorganization with numerous large and irregular cavities, which are apparently blood sinuses. These enlarged cavities support the raised endocranial plate, which is recognized macroscopically as the remodeled *overgrowth* called HFI. Finally, the inner plate totally disappears; the reorganized bone expands towards the diploic space and the cranial cavity, while only a thin shell of lamellar bone remains to envelope the bulbous cavity. According to the Global Model, neither the external plate nor the spongiosa are directly involved since the bulging of the inner plate is primarily occurring *due to newly formed lamellar bone produced by the endosteal dura*. This theory could be greatly supported by the recent micro-CT study where the inner table of females with HFI showed higher porosity compared with females without HFI, possibly occurring as a result of penetration of blood vessels from the dura [14].

1.7 Etiology of HFI

Despite the vast research into HFI, every discussion on the etiology of this phenomenon mostly relies on correlative rather than causal factors [34]. A review of the clinical literature has shown that HFI has been associated with many conditions, including headaches, psychoneurosis, virilism, obesity, pregnancy, acromegaly and

diabetes [19,35–38]. HFI has been included in the Morgagni's syndrome (HFI, obesity, virilism), Stewart-Morel syndrome (HFI, obesity, mental disturbances) and Troell-Junet syndrome (HFI, acromegaly, toxic goiter and diabetes mellitus) [4,35,36,39]. However, these associations were mostly based on case reports and several studies found no significant differences in the association of these factors with HFI versus control groups [35,40,41]. HFI is now viewed as an independent entity, rather than part of a syndrome, since its only clear association is with elderly postmenopausal women [24]. HFI is common in elderly females and therefore may be associated with many common diseases (e.g. with osteoporosis, sterility, diabetes) [7]. Some authors even suggest that old age and longevity could be a primary factor in HFI etiology [8].

The obvious difference in sex distribution of this phenomenon led to the conclusion that some sort of dysendocrinism could be the most plausible cause of HFI. Hershkovitz et al. suggested that the functional disturbance of the gonads, i.e. faulty estrogen stimulation or abnormal progesterone effect on the ovaries or inadequate androgen stimulation by the testis, is the main cause of HFI [7]. In this regard, the research of Kollin et al.[42] is frequently cited – they have shown that the analysis of hormones, including dehydroepiandrosterone, dehydroepiandrosterone sulphate, testosterone and 17α-hydroxy-progesterone, revealed an increase of serum levels in premenopausal females with HFI in comparison to healthy females. However, it is still debatable whether steroid hormone increase, decrease or their disturbed correlation might be responsible for HFI pathogenesis. In males, a low androgen level seems to be an important correlating factor for the origin of the condition [17], while in females, the correlation seems to be more complex.

Since estrogen is considered the main steroid hormone for inducing bone formation in both sexes [43–46], the first association with HFI and hormones was estrogen surplus [7,17,18,42]. Indeed, it has been shown on animal models (mice) that injections of estradiol produced bone changes similar to those seen in HFI [47]. This theory was also supported by the fact that HFI was rare in the past populations, when women spent much of their reproductive period either pregnant or nursing, which implied minimal estrogen exposure [7]. There is a prevalent opinion among anthropologists that the change in HFI prevalence during the last century may be linked to an alteration in hormonal levels due to changes in lifestyle, a dramatic decrease in the

number of children, shorter periods of breast feeding, extended reproductive period and early age onset of menarche [7,8,13,48]. Hormonal alteration in modern females also may occur as a result of hormonal manipulation (i.e. contraceptives, hormonal replacement therapy) and changed diet (consumption of meat contaminated with hormones and phytoestrogens from sources such as soy, grains, etc.) [49–52].

HFI is also found co-occurring with hyperprolactinemia [37], increased leptin [53], progesterone [42] and growth hormone [37]. Western et al. suggest that increased androgen levels (mostly free testosterone) are likely to play a pivotal role in the development of HFI in females (particularly of postmenopausal age) in conjunction with the "gonadotrophic" effect of insulin and IGF-1 associated with obesity and hyperinsulinemia; the results of their study suggest that nulliparity co-occurs with HFI, but is not a primary factor in its pathogenesis [8]. Even a disturbance of the tuberoinfundibular portion of the pituitary gland was considered an etiologic factor of HFI [31]; in our opinion, such condition would have to have a more serious systemic presentation.

Finally, there is a premise that HFI could be a genetically determined illness [21]. The theory was proposed after a few observations of HFI occurring repeatedly in several generations of one family (in one research, in five women from a three-generation family [54]). It is unlikely that an "HFI-associated gene" would ever be discovered. On the other hand, maybe Perou was right stating that cranial hyperostosis "needs a given soil to start (genetic predisposition?) and a given stimulus to manifest itself (external trigger?)" [6].

1.8 HFI in males

Due to the fact that HFI is much more common in females than in males, studies on HFI in male populations are sparse, mostly in a form of case reports (Table 1 summarizes the cases where the medical history of subjects was known). To our knowledge, there is only one study with experimental design where the authors examined the association between androgen deprivation and development of HFI in males [17]. It was found that males who had received a complete androgen block (as a treatment for prostate cancer) manifested a significantly higher prevalence of HFI compared to healthy males. Also, a positive association between the length of hormonal

treatment and manifestation of HFI was shown – the longer the duration of hormonal treatment, the higher the risk of developing HFI. It seems that severe cases of HFI may be found only in males who suffered from hypogonadism, either relative or absolute.

Table 1. Case reports describing HFI in males.

Author	**Case description**
Herschkovitz [7]	Age not specified; single atrophic testis present
Ramchandren [9]	30 yo; obese, Klinefelter's syndrome (47, xxy)
Néel [55]	Age not specified; Klinefelter's syndrome (47, xxy)
Yamakawa [56]	72 yo; primary (hypergonadotropic) hypogonadism
Belcastro [20]	78 yo; prepubertal castration (the case of famous singer Farinelli)
Miazgowski [57]	36 yo; Kallman's syndrome (hypogonadotropic hypogonadism)

Hypogonadism is a condition characterized by either testosterone deficiency or defective spermatogenesis and often both disorders coexist. Male hypogonadism may be classified into three categories according to the level of the defect. Diseases directly affecting the testes result in *primary* or *hypergonadotropic hypogonadism,* characterized by oligospermia/azoospermia and low testosterone levels, but with elevations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) because of a decrease of the negative feedback regulation on the pituitary and hypothalamus by androgens, estrogens and inhibin. *Secondary* or *hypogonadotropic hypogonadism,* which occurs due to lesions in the hypothalamus or pituitary gland, is characterized by a low testosterone level or ineffective spermatogenesis as the result of inadequate stimulation of the testes by insufficient or inadequate concentrations of gonadotropins. The third category of hypogonadism is the result of defects in androgen action (e.g. lack of androgen receptor) [58]. On the other hand, hypogonadism in males can also be *relative*, meaning that spermatogenesis and testosterone production are adequate, but metabolism of androgens is altered (e.g. changes in testosterone metabolism in liver cirrhosis, obesity, etc.).

Some researchers argue that it may be possible that androgen alteration itself does not produce the HFI phenomenon, but rather the change in the estrogen/androgen ratio (namely, a surplus of estrogen) [17].

1.9 Bone formation in HFI – a localized or systemic phenomenon?

If hormonal imbalance was considered to be a probable cause of HFI occurrence, it would be expected that the changes in hormonal levels have some impact on other sites, especially concerning the skeletal system. This is the question that many researchers have proposed, although there are only few studies where the hypothesis was tested. Kollin et al. [42] tried to determine whether bone mineral content and bone width of the radius differ between premenopausal woman with HFI and their age-matched controls. In their study sample, the results showed that the females with HFI had greater than normal bone mineral content, bone width of the radius and bone mineral content to bone width ratio. Based on that, these authors considered that HFI could not be an isolated phenomenon, but rather the most easily detectable radiological sign of a metabolic disorder. On the other hand, bone loss in the postcranial skeleton showed the same pattern in postmenopausal females with HFI as in those without HFI [59].

Studies have shown that HFI could, at least, be a *regional* phenomenon affecting the skull bones. Increased bone thickness of the frontal bone affected by HFI is followed by an increase in the thickness of temporal bones [8,14]. Some authors underline that HFI is accompanied by an increase in the thickness of all cranial bones, in a process that is synchronized and leads to a reduced intracranial volume [38].

1.10 The use of HFI in forensic anthropology

In various anthropological and forensic investigations, estimation of sex and age is essential when dealing with skeletal human remains. The validity of the established methods for sexing and aging skulls alone decreases considerably with the individual's age and the need for a sex-linked trait that can be easily identified in forensic cases frequently emerges [48]. The postcranial skeleton (e.g. long bones and pelvis in particular) is considered a better indicator for sex assessment than the skull, which many

authors regard as the second best [60–62]. Physical anthropologists record morphological (nonmetric) traits, some of them binary in nature (present/absent) or represented as ordinal grades of expression [63]. Many skull landmarks are being used for this purpose, such as size differences of the mastoid processes, glabella, supraorbital ridges, palate, frontal sinuses [60–63]. Specific pathological features of the skull, such as healed fractures, previously known interventions (e.g. craniotomy), tumors of the cranial bones or the presence of a unique condition like HFI, could be useful for identification.

In the past decades, when a complete skull was discovered, radiographs and endoscopy were the only diagnostic options for observing the interior of the skull. In practice, however, incomplete skeletons, skulls in isolation or even fragments of the skulls are found. The fact that HFI could be observed when only the frontal bone is preserved could be very helpful for the sex and age determination of the skeletal remains.

2 Research goals

1. To establish whether the occurrence of HFI in males is age-dependent.
2. To investigate the morphology of different HFI types in males and females.
3. To investigate the differences in the size of the cranial vault by measuring longitudinal and transversal diameters of the skulls, as well as the thickness of the frontal, temporal and occipital bones between males and females with and without HFI.
4. To investigate and compare the microarchitecture of the frontal bone in males with different types of HFI.
5. To investigate and compare the microarchitecture of the frontal bone between males and females with corresponding types of HFI.
6. To compare femoral bone mineral density, femoral geometry and femoral microarchitecture of males and females with HFI to those without this condition.
7. To investigate femoral bone mineral density, femoral geometry and femoral microarchitecture between males and females with HFI.
8. To establish the possible use of the skull with HFI as an additional criterion for the sex and age identification of human remains.

3 Material and methods

The research comprised two studies. The first part included an observational autopsy study whose aim was to analyze age distribution, macroscopic appearance and morphological features of male skulls with HFI compared to females with this condition. The second study was designed as a case-control study and was carried out on selected, age-matched subjects. Its purpose was to analyze the microarchitectural structure of the affected frontal bone and to examine whether the frontal bone formation (the key feature of HFI) accompanies changes in bone structure in a systemic manner. This was accomplished using microcomputed tomography (micro-CT imaging) on the frontal and femoral bone samples. Before micro-CT imaging of the femoral samples proximal parts of femora were also scanned using dual energy x-ray absorptiometry (DXA).

3.1 Observational autopsy study

Observational, cross-sectional autopsy study was carried out at the Institute of Forensic Medicine, School of Medicine, University of Belgrade, where ethics approval for the collection of the sample was granted by the Ethics Committee (Approval no.2650/XII-14). The sample was taken from human donor cadavers and divided into four groups: study group of males with HFI and three control groups (males without HFI and females with and without HFI). Having recorded every case of HFI (male or female), we randomly selected 1 or 2 cases without this condition (in this part of the study subjects were not age matched). The inclusion criteria for the study were determined age and sex of the subjects, adult population (18 years or older) and Caucasian race. The exclusion criteria included bone-related pathology of the cranium (other than HFI), history of brain or meningeal tumor, skull fractures (due to inability to conduct proper skull measuring), endocrine or metabolic diseases which affect the skeleton (e.g. chronic renal disease, primary hyperparathyroidism, Paget's disease, metastatic malignant disease, liver cirrhosis) and the use of medications known to significantly interfere with bone metabolism (e.g. glucocorticoids, bisphosphonates). In all subjects who met the inclusion criteria, the following variables were recorded: sex, age and HFI type (if present).

3.1.1 *Morphological measurements of the skulls*

During autopsies crania were opened with an electric saw, using the standard technique: around 2 cm above the glabellar region and around 1 cm below the external occipital protuberance. In every subject with HFI, two forensic pathologists independently confirmed the type of HFI using classification based on the macroscopic morphological characteristics of HFI proposed by Herschkovitz et al. [7] (Figure 1): "type A – isolated elevated bony islands, single or multiple, generally under 10 mm in size, found in the anteromedial part of the frontal bone; type B – nodular bony overgrowths with slight elevation, identified on less than 25% of the frontal bone; type C – extensive nodular bony overgrowth with irregular thickening of up to 50% of the frontal endocranial surface; and type D – continuous bony overgrowth, involving more than 50% of the frontal endocranial surface". In all subjects, the thickness of the frontal, temporal and occipital bones (avoiding the middle sagittal line), as well as the longitudinal and frontal diameters of the skulls was measured. Measurements were carried out with a ruler, accurate to 1 mm.

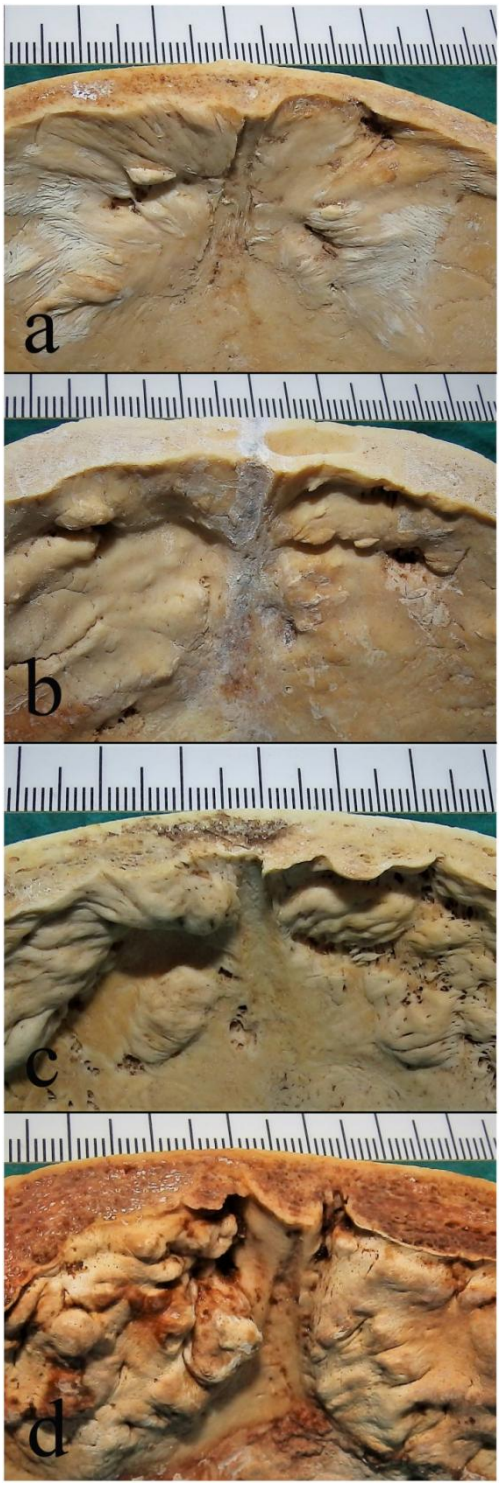

Figure 1. Macroscopic classification of HFI according to Hershkovitz et al [7];
a – type A, male, 84 years old; b – type B, male, 57 years old;
c – type C, male, 75 years old; d – type D, male, 44 years old.

3.2 Case-control study

3.2.1 *Specimen harvesting*

The selected subjects were age-matched and bone samples were taken in all four sample groups. The specimens of the frontal bone were collected only from the subjects with HFI (male subjects were cases and female subjects were controls). The specimens of the proximal femur were collected from all selected subjects (subjects with HFI were cases and the ones without were controls).

Frontal bone samples (1x1 cm) were harvested using a slowly rotating electric saw from the part where the frontal bone was the thickest and affected by hyperostosis. The samples were manually cleaned of adherent soft tissue, dried and stored for the microarchitectural analysis (micro-CT imaging).

The proximal parts of femora were harvested with the electric saw, manually cleaned of adherent soft tissue and left to dry at room temperature for several weeks. Residual soft tissue and bone marrow were removed by cooking in water with cationic detergent for several hours. Finally, the samples were bleached using 10% hydrogen peroxide. After DXA scans were done, bone samples (1x1 cm) were taken from the lateral region of the femoral neck (Figure 2) for further microarchitectural analysis (micro-CT imaging), using the slowly rotating electric saw.

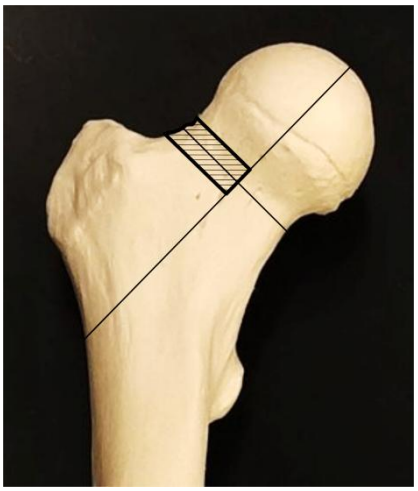

Figure 2. The region of the femoral neck taken for the microarchitectural analysis.

3.2.2 *Dual energy x-ray absorptiometry and hip structure analysis*

Bone densitometry was measured using dual energy x-ray absorptiometry scans *ex vivo* on the proximal femora (Hologic QDR 1000/W, Waltham, MA). Femoral specimens were placed in an antero-posterior position and submerged in a water bath to simulate soft tissue [64]. The conventional DXA scan software measures bone mineral density as the average value of pixels within an image region of interest, after excluding pixels below a certain threshold [65]. The scans were automatically evaluated by DXA software which provided values of bone area (BA; cm^2), bone mineral content (BMC; g) and areal bone mineral density (BMD; g/cm^2) in total femoral sample and neck region. BMD is measured as the mean pixel value and BA as the sum of the pixel areas. BMC (g) is then computed as the product of BA (cm^2) and BMD (g/cm^2).

Hip structure analysis (HSA) software, developed by Beck and colleagues [66], uses both the dimensional information and the mineral mass data derived from DXA scans to compute the types of dimensional properties of the scanned samples. In engineering analysis, a similar method incorporates dimensions of the object and information on the directions and magnitude of applied forces to compute stresses at likely failure points [65,66]. HSA software is implemented on DXA Hologic software version 2.0 and in this research it was used to estimate *femoral geometry*. The following three regions of interest corresponding to 5-mm-thick cross-sectional slabs of bone were assessed in this analysis (Figure 3): narrow neck (NN) located at the narrowest diameter of the femoral neck; the intertrochanteric (IT) at the level of the section of femoral neck and shaft axes; and the shaft (FS) located 1.5 times the neck width distal to the intersection of the neck and shaft axes. The following structural parameters were calculated for each region of interest: cross-sectional area (CSA, cm^2), section modulus (SM, cm^3), estimated cortical thickness (CTh, cm) and estimated buckling ratio (BR, dimensionless). These parameters are defined as follows [65,66]:

- *Cross sectional area* represents bone resistance to compressive load. It refers to the surface of bone tissue after subtracting the area of voids, spaces, and marrow cavity which do not provide significant load support.

- *Section modulus* is a measure of bending and torsional strength of the bone. It is computed as the cross-sectional moment of inertia divided by the maximum distance to the medial or lateral profile margin.
- *Estimated cortical thickness* is calculated as the difference between periosteal and endocortical diameter of the bone. Periosteal diameter is measured directly from DXA scans, while endocortical diameter is derived from the mathematical model.
- *Buckling ratio* indicates bone instability due to thinning of the cortical bone. It is calculated when the half of the periosteal diameter is divided by cortical thickness.

In order to avoid the influence of height and weight on the result of densitometric and HSA measurements, we have recorded height and weight of all the subjects who underwent DXA scans and adjusted data in the statistical analysis.

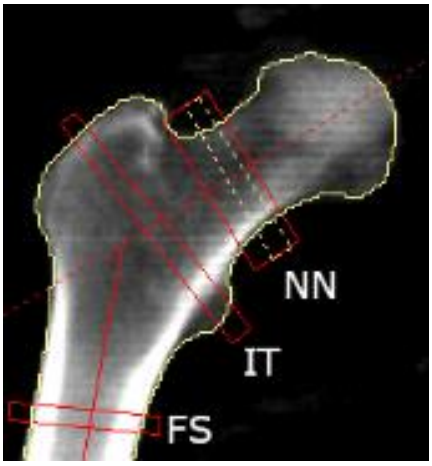

Figure 3. DXA scan image of the right femur showing regions of interest used in hip structure analysis (NN – narrowest neck, IT– intertrochanteric region, FS – femoral shaft region).

3.2.3 *Microcomputed tomography (micro-CT) imaging*

The specimens of the frontal bone and femur were scanned using microcomputed tomography (SkyScan 1172 μCT) in the Laboratory for Anthropology, Institute of Anatomy, School of Medicine, University of Belgrade. Each bone specimen sample was placed in a sample holder with a consistent orientation and scanned in dry conditions. The micro-CT was operated at 80 kV, 124 μA and 1200 μs exposure time, with an isotropic resolution of 10 μm and applying Al+Cu filter. The microarchitecture of bone was evaluated automatically using micro-CT evaluation program *CT.An* with direct 3D morphometry. The threshold was set at 110/255 for frontal bone samples and 85/255 for femoral samples.

Microarchitectural analysis was conducted on frontal bones samples belonging to subjects who had HFI and were previously selected for further analysis (DXA and HAS scans). In those, we defined four regions of interest in the frontal bone sample: total sample, outer table (*tabula externa*), spongiosa and inner table (*tabula interna*). Microarchitectural analysis was also done on femoral neck samples in all subjects who underwent DXA and HAS scans (males and females with and without HFI); regarding these we set two regions of interest: trabecular and cortical bone. The following microarchitectural parameters were determined: bone volume fraction (BV/TV, %), pore diameter (Po.Dm, mm), pore separation (Po.Sp, mm), closed porosity (Po.Cl, %), open porosity (Po.Op, %), total porosity (Po.Tot, %), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), fractal dimension (FD), structure model index (SMI, dimensionless), connectivity density (Conn.D, 1/mm3) and degree of anisotropy (DA). These parameters are defined as follows [67–69]:

- *Bone volume fraction* is the ratio of the segmented bone volume to the total volume of the region of interest.
- *Pore diameter* is a mean diameter of cortical pores, assessed using direct 3D methods.
- *Pore separation* is a mean distance between cortical pores, assessed using direct 3D methods.

- *Porosity analysis* refers to the counting of empty spaces and characterization of their connections. If an empty space is fully surrounded by material (bone) on all sides, it is referred to as *closed porosity*. If the empty space is not fully enclosed, but at some point it can find a connection to the space outside of the object, it is defined as *open porosity*.
- *Trabecular number* is a measure of the average number of trabeculae per unit length.
- *Trabecular thickness* is a mean thickness of trabeculae, assessed using direct 3D methods.
- *Trabecular separation* is a mean distance between trabeculae, assessed using direct 3D methods.
- *Fractal dimension* is used to assess the pattern of the trabecular bone in digital imaging. It is based on the fractal analysis as mathematical method that numerically describes complex shapes and structural patterns.
- *Structure model index* is a measure of the relative proportion of plate-like vs. rod-like trabeculae in a given region. Values range from 0 (idealized plates) to 3 (idealized rods) and can be positive or negative. Negative values indicate more concave or closed structure, like a honeycomb; positive values indicate a more convex and open structure.
- *Connectivity density* is a measure of the degree of connectivity of trabeculae normalized by the total volume of the region of interest.
- *Degree of anisotropy* is a measure of the structural anisotropy (1= isotropic, > 1 = anisotropic by definition).

3.3 Statistical analysis

Sample size was calculated in MedCALC statistical software (version 18.10), taking into consideration the expected variability of the investigated parameters and biologically relevant level of differences intended to be detected, with type I error of 0.05 and type II error of 0.20. Kolmogorov-Smirnov test was applied to assess the normality of the subjects' age data distribution, skull morphological features (thickness of frontal, temporal and occipital bones, longitudinal and frontal diameters), densitometric, HSA and microarchitectural parameters. Parametric tests (Student's t-

test, one-way analysis of variance – ANOVA) in the case of normal distribution or appropriate non-parametric tests (Mann-Whitney test, Kruskal-Wallis test) were used for assessing the differences in mean values of the abovementioned variables between selected groups. In case of post-hoc ANOVA analysis, Tukey test was used. Chi square was used to compare the differences in the distribution of HFI subtypes. Logistic regression was implemented to establish whether HFI is an age-related phenomenon and the ROC curve was applied for estimating cut-off values (in years) for HFI occurrence. Spearman's correlation was used to test the correlation between HFI severity and age, skull bones thickness and DXA parameters and also to test the correlation between the presence/severity of HFI (without HFI, milder, severe) and microarchitectural parameters of the femoral bone. All obtained densitometric and HSA data were adjusted for body height and weight using univariate linear model, in order to avoid the influence of these parameters on the results (given that the dimensions of the femur, as well as its cross-sectional properties depend, at least partially, on bone size and age). The tests were performed in the SPSS statistical package (version 20.0) with 0.05 significance level.

4 Results

4.1 Observational autopsy study

4.1.1 *Characteristics of the study sample*

The study sample comprised 41 males with HFI, 55 males without HFI, 119 females with HFI and 202 females without HFI. Table 2 shows age distribution of the study population. There was no significant age difference between the males with and without HFI ($t = -0.225$; $df=94$; $p>0.05$), but the females with HFI were older than the males with HFI and females without HFI (Mann Whitney $U=1413.500$, $p<0.05$; Mann Whitney $U=6869.000$, $p<0.05$, respectively). In our sample, age seemed to be a predicting factor for HFI occurrence in females, but not in males. On the other hand, in females below 55, age did not determine HFI occurrence (Table 3). The cut-off value for HFI occurrence in females was 68.5 years, with a sensitivity of 73% and specificity of 63% (AUC=0.714; 0.658-0.771; $p<0.05$).

Table 2. Age distribution of the study population.

	Males with HFI	**Males without HFI**	**Females with HFI**	**Females without HFI**
Mean ± SD (years)	62 ± 16	61 ± 17	72 ± 14	58 ± 20
Range (years)	27 – 85	24 – 93	19 – 93	18 – 101

Table 3. Correlation between age and HFI occurrence, regarding sex.

Age	**B**	**p-value**	**OR**	**CI95%**
Females	0.047	**0.001***	1.048	1.031-1.064
Females <60 yrs	0.051	**0.020***	1.052	1.008-1.098
Females <55 yrs	0.023	0.376	1.024	0.972-1.078
Males	0.003	0.820	1.003	0.979-1.028

B – coefficient*; OR* – odds ratio; *CI* – confidence interval

* $p<0.05$; univariate logistic regression model

The most common HFI type among males was type B and among females type C (χ^2=12.549; df=3; p<0.01). The severity of HFI did not correlate with age either in males or females (for males, Spearman's Rho = 0.068, N=41, p>0.05; for females Spearman's Rho = 0.173, N=119, p>0.05). Tables 4 and 5 show the distribution of HFI subtypes according to age intervals in both sexes. Severe HFI (type C and D) is 3.5 times more common in females than in males (OR=3.536, CI 95% 1.675-7.463, p<0.05).

Table 4. Distribution of HFI subtypes according to age intervals, in males.

Males		**Age interval (years)**							Total
		21-30	31-40	41-50	51-60	61-70	71-80	>80	
HFI	**A**	2	2	0	0	2	2	0	8 (20%)
type	**B**	0	1	2	1	8	4	3	19 (46%)
	C	0	0	1	5	2	1	1	10 (24%)
	D	0	0	1	0	2	1	0	4 (10%)
Total		2	3	4	6	14	8	4	41 (100%)

Table 5. Distribution of HFI subtypes according to age intervals, in females.

Females		**Age interval (years)**								Total
		18-20	21-30	31-40	41-50	51-60	61-70	71-80	>80	
HFI	**A**	1	1	1	0	3	2	4	4	16 (13%)
type	**B**	0	0	1	2	4	4	10	5	26 (22%)
	C	0	1	0	0	4	9	23	11	48 (40%)
	D	0	0	1	0	3	5	9	11	29 (25%)
Total		1	2	3	2	14	20	45	31	119 (100%)

4.1.2 *Morphological measurements of the skulls*

Tables 6 and 7 show the morphologic features of skulls in the study sample. Frontal and temporal bones are thicker in subjects that have HFI, regardless of sex (in males, for the frontal bone $t = -9.145$; $df=94$; $p<0.05$, and for the temporal bone $t = -4.552$; $df=94$; $p<0.05$; in females, for the frontal bone Mann Whitney $U=2471.500$, $p<0.05$, and for the temporal bone $U=6346.500$, $p<0.05$). Females with HFI also have thicker occipital bone and smaller longitudinal diameter of the skull, compared to females without HFI (Mann Whitney $U=9793.500$, $p<0.05$; Mann Whitney $U=9793.500$, $p<0.05$; Mann Whitney $U=10007.500$, $p<0.05$). Males with HFI tend to have thicker occipital bone compared to males without HFI, but this difference reached only borderline significance ($p=0.069$).

Table 6. Thickness of the frontal, temporal and occipital bones in the study sample.

	Frontal (mm)	**Temporal (mm)**	**Occipital (mm)**
Males with HFI	10 ± 2.2	7 ± 1.8	8.2 ± 2.2
Males without HFI	6.3 ± 1.7	5.3 ± 1.8	7.5 ± 1.4
p-value	**0.001***	**0.001***	**0.069**
Females with HFI	9.9 ± 2.9	6.4 ± 2.2	7.3 ± 2.0
Females without HFI	5.5 ± 1.9	4.8 ± 1.4	6.7 ± 1.7
p-value	**0.001***	**0.001***	**0.005***

* $p<0.05$

Table 7. Longitudinal and frontal diameter of the skulls in the study sample.

	Longitudinal diameter (mm)	**Frontal diameter (mm)**
Males with HFI	155.1 ± 9.3	138.2 ±7
Males without HFI	154.2±6.9	139.2±7.5
p-value	0.601	0.497
Females with HFI	149.8 ± 6.8	133.7 ± 6.2
Females without HFI	151.8 ± 6.8	133.6 ± 6.4
p-value	**0.012***	0.529

* $p<0.05$

In subjects with HFI, the thickness of the frontal bone was not correlated with sex. However, thicker temporal or occipital bone was a predictive factor for the male sex (Table 8); the cut-off values were 6.5 mm for temporal (AUC=0.613; 0.516-0.709; $p<0.05$) and 7.5 mm for the occipital bone (AUC=0.625; 0.524-0.726; $p<0.05$), although the Spearman's coefficient showed poor correlation. There was no correlation between the thickness of the skull bones and the age of subjects with HFI (Table 8). The correlations were found between the thickness of the frontal and temporal bones and the severity of HFI in males. In females, such correlation was found only for the frontal bone (Table 9).

Table 8. The correlation between sex, age and skull bone thickness in subjects with HFI.

	Frontal bone	**Temporal bone**	**Occipital bone**
Sex (N = 160)			
Spearman's Rho	-0.032 [a]	-0.173 [a]	-0.191 [a]
p-value	0.692	**0.029***	**0.015***
Age (N = 160)			
Spearman's Rho	0.077	0.043	-0.129
p-value	0.332	0.590	0.140

* $p<0.05$

[a] male sex coded as 0, female as 1

Table 9. The correlation between severity of HFI and skull bone thickness.

	Frontal bone	**Temporal bone**	**Occipital bone**
Males (N = 41)			
Spearman's Rho	0.595**	0.309**	0.096
p value	**0.000***	**0.049***	0.551
Females (N = 119)			
Spearman's Rho	0.405**	0.159	0.161
p value	**0.000***	0.085	0.080

* $p<0.05$

** Spearman's Rho >0.3 is considered a good correlation

4.1.3 *Applications in forensic anthropology*

Combining the results from the observational autopsy study, here we want to summarize the possible use of HFI presence in forensic anthropology, concerning sex and age determination of skeletal remains.

The results of our study have shown that severe form of HFI (type C and D) is 3.5 times more common in females than in males (OR=3.536, CI 95% 1.675-7.463, $p<0.05$). The severity of HFI did not correlate with age either in males or females (for males, Spearman's Rho = 0.068, N=41, $p>0.05$; for females Spearman's Rho = 0.173, N=119, $p>0.05$). However, in females, age seemed to be a predicting factor for HFI occurrence. The cut-off value for HFI occurrence in females was 68.5 years, with a sensitivity of 73% and specificity of 63% (AUC=0.714; 0.658-0.771; $p<0.05$).

The thickness of the frontal bone was not correlated with sex, either in males or females. On the other hand, thicker temporal or occipital bone was a predictive factor for the male sex (Table 8); the cut-off values were 6.5 mm for temporal (AUC=0.613; 0.516-0.709; $p<0.05$) and 7.5 mm for the occipital bone (AUC=0.625; 0.524-0.726; $p<0.05$), although the Spearman's coefficient showed poor correlation. There was no correlation between the thickness of the skull bones and the age of subjects with HFI (Table 8). The correlations were found between the thickness of the frontal and temporal bones and the severity of HFI in males. In females, such correlation was found only for the frontal bone (Table 9).

4.2 Case-control study

4.2.1 *Densitometric measurements*

The femora from 36 males (19 with and 19 without HFI) and 34 females (17 with and 17 without HFI) were selected for further DXA scans and HSA analysis. They were age-matched within the same sex, which is represented in Table 10.

Table 10. Age distribution in males and females selected for DXA and HAS analysis.

	Males with HFI (N = 19)	**Males without HFI (N = 19)**	**Females with HFI (N = 17)**	**Females without HFI (N = 17)**
Mean ± SD (years)	60 ± 15	62 ± 16	75 ± 15	74 ± 16
Range (years)	27 – 85	29 – 84	28 – 88	23 – 90

Unadjusted and adjusted data revealed no significant statistical difference in densitometric measurements between the group with and without HFI (Tables 11 and 12) in either sex. As expected, unadjusted data between males and females with HFI showed significant statistical difference in favor of males in every measured parameter. After adjustment for age, height and weight, the difference remained significant for bone area ($F=12.173$, $df=1$, $p<0.05$) and mineral content in the neck region ($F=7.071$, $df=1$, $p<0.05$) (Table 13).

Table 11. DXA analysis of males with and without HFI, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=177.68 cm; weight=87.26 kg).

DXA parameter before adjusting	**Males with HFI (Mean ± SD)**	**Males without HFI (Mean ± SD)**		**p value**
BA n (cm^2)	5.73 ± 0.84	5.41 ± 0.92		0.265
BA t (cm^2)	53.50 ± 6.75	53.41 ± 8.97		0.973
BMC n (g)	4.68 ± 1.28	4.14 ± 1.04		0.160
BMC t (g)	55.00 ± 15.81	52.72 ± 9.44		0.231
BMD n (g/cm^2)	0.82 ± 0.19	0.76 ± 0.11		0.277
BMD t (g/cm^2)	1.04 ± 0.21	0.95 ± 0.13		0.152
DXA parameter after adjusting	**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
BA n (cm^2)	5.73 ± 0.21	5.41 ± 0.21	0.376	0.301
BA t (cm^2)	52.73 ± 1.84	54.18 ± 1.84	0.409	0.591
BMC n (g)	4.60 ± 0.30	4.21 ± 0.30	0.123	0.314
BMC t (g)	54.69 ± 2.98	52.00 ± 2.98	0.170	0.537
BMD n (g/cm^2)	0.80 ± 0.04	0.77 ± 0.04	0.104	0.576
BMD t (g/cm^2)	1.03 ± 0.04	0.91 ± 0.04	0.258	0.321

n – femoral neck region; *t* – total femoral sample

Table 12. DXA analysis of females with and without HFI, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=161.85 cm; weight=62.56 kg).

DXA parameter before adjusting	**Females with HFI (Mean ± SD)**	**Females without HFI (Mean ± SD)**		**p value**
BA n (cm^2)	4.54 ± 1.16	4.98 ± 1.08		0.270
BA t (cm^2)	47.98 ± 5.26	49.16 ± 6.52		0.563
BMC n (g)	2.75 ± 0.93	3.10 ± 1.16		0.340
BMC t (g)	39.09 ± 9.19	40.29 ± 10.80		0.728
BMD n (g/cm^2)	0.61 ± 0.15	0.62 ± 0.17		0.916
BMD t (g/cm^2)	0.81 ± 0.17	0.81 ± 0.16		0.978
DXA parameter after adjusting	**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
BA n (cm^2)	4.52 ± 0.28	4.99 ± 0.28	0.639	0.265
BA t (cm^2)	48.09 ± 1.52	49.05 ± 1.52	0.662	0.909
BMC n (g)	2.71 ± 0.25	3. 41 ± 0.25	0.119	0.234
BMC t (g)	38.84 ± 1.96	40.54 ± 1.96	0.002*	0.550
BMD n (g/cm^2)	0.61 ± 0.03	0.63 ± 0.03	0.001*	0.639
BMD t (g/cm^2)	0.81 ± 0.03	0.82 ± 0.03	0.001*	0.706

n – femoral neck region; *t* – total femoral sample

* $p<0.05$

Table 13. DXA analysis of males and females with HFI, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: age=67.49 years; height=171.44 cm; weight=79.06 kg).

DXA parameter before adjusting	**Males with HFI (Mean ± SD)**	**Females with HFI (Mean ± SD)**		**p value**
BA n (cm^2)	5.73 ± 0.84	4.54 ± 1.16		0.002*
BA t (cm^2)	53.50 ± 6.75	47.98 ± 5.26		0.010*
BMC n (g)	4.68 ± 1.28	2.75 ± 0.93		0.000*
BMC t (g)	55.00 ± 15.81	39.09 ± 9.19		0.000*
BMD n (g/cm^2)	0.82 ± 0.19	0.61 ± 0.15		0.001*
BMD t (g/cm^2)	1.04 ± 0.21	0.81 ± 0.17		0.002*
DXA parameter after adjusting	**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
BA n (cm^2)	6.07 ± 0.31	4.16 ± 0.33	0.006*	0.001*
BA t (cm^2)	53.20 ± 1.89	48.30 ± 2.06	0.059	0.159
BMC n (g)	4.57 ± 0.36	2.88 ± 0.39	0.001*	0.013*
BMC t (g)	52.72 ± 4.06	42.74 ± 4.41	0.005*	0.179
BMD n (g/cm^2)	**0.74 ± 0.05**	**0.69 ± 0.05**	**0.000***	**0.625**
BMD t (g/cm^2)	**0.97 ± 0.06**	**0.89 ± 0.06**	**0.001***	**0.423**

n – femoral neck region; *t* – total femoral sample

* $p<0.05$

4.2.2 *Hip structure analysis*

HSA could not be done in all marked femoral regions (neck, intertrochanteric, shaft) due to the blurriness of the sample edges or incomplete images retrieved from DXA scans (Table 14).

Table 14. Missing data in HSA analysis.

HSA parameter	CSA NN	CSA IT	CSA FS	SM NN	SM IT	SM FS	CTh NN	CTh IT	CTh FS	BR NN	BR IT	BR FS
Missing data (%)	5.6	4.2	0	0	5.6	4.2	0	6.9	13.9	16.7	22.2	20.8

Cross-sectional area decreased in the neck and increased in the intertrochanteric region among males with HFI, compared to males without this condition. After adjustment for height and weight, these differences remained valid ($F=102.911$, $df=1$, $p<0.05$; $F=4.557$, $df=1$, $p<0.05$; Table 15). In females, there were no differences in HSA parameters between the groups with and without HFI, before and after adjustment of data for height and weight (Table 16). In the group of subjects with HFI, statistical differences were detected between sexes with unadjusted data, regarding cross-sectional area in the neck and intertrochanteric region, section modulus in all three regions and estimated cortical thickness in the neck region. However, after adjusting for height and weight, the only remaining statistical difference was the one with section modulus in the neck region ($F=7.699$, $df=1$, $p<0.05$; Table 17).

Table 15. HSA in males with and without HFI, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=177.80 cm; weight=79.54 kg).

HSA parameter before adjusting	Region	Males with HFI (Mean ± SD)	Males without HFI (Mean ± SD)		p value
CSA (cm^2)	**NN**	**3.49 ± 0.86**	**6.61 ± 1.51**		**0.000***
	IT	**7.86 ± 4.20**	**5.06 ± 0.93**		**0.011***
	FS	4.92 ± 2.15	3.85 ± 1.15		0.065
SM (cm^3)	NN	2.25 ± 0.63	1.90 ± 0.42		0.052
	IT	9.12 ± 5.20	6.88 ± 2.22		0.103
	FS	3.12 ± 1.47	3.05 ± 0.63		0.879
CTh (cm)	NN	0.18 ± 0.04	0.17 ± 0.01		0.383
	IT	0.67 ± 0.37	0.89 ± 0.74		0.345
	FS	0.59 ± 0.26	0.79 ± 0.51		0.164
BR	NN	12.24 ± 3.86	11.94 ± 2.59		0.784
	IT	6.58 ± 3.03	7.75 ± 1.53		0.183
	FS	3.07 ± 2.51	2.72 ± 0.60		0.593
HSA parameter after adjusting		**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
CSA (cm^2)	**NN**	**3.28 ± 0.23**	**6.89 ± 0.26**	**0.000***	**0.000***
	IT	**7.64 ± 0.72**	**5.33 ± 0.79**	**0.017***	**0.044***
	FS	4.67 ± 0.36	4.10 ± 0.36	0.005*	0.288
SM (cm^3)	NN	2.17 ± 0.11	1.97 ± 0.11	0.007*	0.227
	IT	8.56 ± 0.89	7.58 ± 1	0.025*	0.484
	FS	2.96 ± 0.26	3.22 ± 0.29	0.237	0.534
CTh (cm)	NN	0.18 ± 0.01	0.18 ± 0.01	0.152	0.696
	IT	0.66 ± 0.14	0.88 ± 0.15	0.770	0.330
	FS	0.80 ± 0.10	0.80 ± 0.10	0.167	0.126
BR	NN	12.33 ± 0.73	11.85 ± 0.73	0.163	0.655
	IT	6.54 ± 0.59	7.80 ± 0.66	0.314	0.714
	FS	3.07 ± 0.46	2.73 ± 0.50	0.791	0.663

* $p<0.05$

Table 16. HSA in females with and without HFI, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=161.85 cm; weight=74.26 kg).

HSA parameter before adjusting	Region	Females with HFI (Mean ± SD)	Females without HFI (Mean ± SD)		p value
CSA (cm^2)	NN	2.34 ± 0.51	5.19 ± 1.24		0.741
	IT	5.06 ± 2.23	3.89 ± 0.69		0.845
	FS	3.77 ± 1.21	2.41 ± 0.75		0.724
SM (cm^3)	NN	1.20 ± 0.31	1.28 ± 0.36		0.462
	IT	5.71 ± 2.77	5.31 ± 1.53		0.603
	FS	2.13 ± 0.78	2.28 ± 0.35		0.463
CTh (cm)	NN	0.15 ± 0.04	0.14 ± 0.04		0.875
	IT	0.44 ± 0.30	0.49 ± 0.27		0.545
	FS	0.49 ± 0.23	0.51 ± 0.13		0.719
BR	NN	13.31 ± 6.35	14.37 ± 5.71		0.614
	IT	8.16 ± 3.78	9.84 ± 2.79		0.150
	FS	3.35 ± 1.72	3.33 ± 1.09		0.962
HSA parameter after adjusting		**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
CSA (cm^2)	NN	2.32 ± 0.11	2.43± 0.11	0.001*	0.461
	IT	4.99 ± 0.39	5.25 ± 0.39	0.648	0.020
	FS	3.76 ± 0.22	3.89 ± 0.22	0.051	0.670
SM (cm^3)	NN	1.18 ± 0.07	1.29 ± 0.07	0.271	0.021
	IT	5.67 ± 0.50	5.36 ± 0.50	0.060	0.670
	FS	2.12 ± 0.15	2.29 ± 0.15	0.690	0.457
CTh (cm)	NN	0.15 ± 0.01	0.15 ± 0.01	0.001*	0.880
	IT	0.43 ± 0.07	0.51 ± 0.07	0.227	0.406
	FS	0.52 ± 0.05	0.49 ± 0.04	0.125	0.675
BR	NN	13.63 ± 1.29	14.06 ± 1.29	0.017*	0.817
	IT	8.29 ± 0.78	9.71 ± 0.78	0.084	0.218
	FS	3.44 ± 0.32	3.25 ± 0.32	0.042*	0.780

* $p<0.05$

Table 17. HSA in males and females with HFI before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: age=67.47 years; height=171.44 cm; weight=79.06 kg).

HSA parameter before adjusting	Region	Males with HFI (Mean ± SD)	Females with HFI (Mean ± SD)		p value
CSA (cm^2)	NN	3.49 ± 0.86	2.34 ± 0.51		0.000*
	IT	7.86 ± 4.20	5.06 ± 2.23		0.017*
	FS	4.92 ± 2.15	3.77 ± 1.21		0.060
SM (cm^3)	NN	2.25 ± 0.63	1.20 ± 0.31		0.000*
	IT	9.12 ± 5.20	5.71 ± 2.77		0.019*
	FS	3.12 ± 1.47	2.13 ± 0.78		0.019*
CTh (cm)	NN	0.18 ± 0.04	0.15 ± 0.04		0.016*
	IT	0.67 ± 0.37	0.44 ± 0.30		0.051
	FS	0.59 ± 0.26	0.49 ± 0.23		0.231
BR	NN	12.24 ± 3.86	13.31 ± 6.35		0.573
	IT	6.58 ± 3.03	8.16 ± 3.78		0.173
	FS	3.07 ± 2.51	3.35 ± 1.72		0.701
HSA parameter after adjusting		**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
CSA (cm^2)	NN	3.25 ± 0.21	2.61 ± 0.23	0.000*	0.105
	IT	6.68 ± 1.01	6.39 ± 1.09	0.023*	0.882
	FS	4.23 ± 0.54	4.53 ± 0.58	0.870	0.750
SM (cm^3)	**NN**	**2.11 ± 0.15**	**1.35 ± 0.17**	**0.000***	**0.011***
	IT	7.19 ± 1.26	7.86 ± 1.36	0.026*	0.767
	FS	2.87 ± 0.38	2.39 ± 0.41	0.127	0.486
CTh (cm)	NN	0.17 ± 0.01	0.16 ± 0.01	0.003*	0.986
	IT	0.74 ± 0.11	0.41 ± 0.12	0.267	0.133
	FS	0.49 ± 0.07	0.61 ± 0.08	0.047*	0.331
BR	NN	14.11 ± 1.45	11.22 ± 1.57	0.042*	0.275
	IT	6.56 ± 1.05	8.18 ± 1.14	0.262	0.397
	FS	3.42 ± 0.65	2.96 ± 0.70	0.248	0.698

* $p<0.05$

4.2.3 *Microarchitectural analysis*

4.2.3.1 Microarchitectural structure of the frontal bone

The distribution of HFI types and age of subjects selected for this analysis are presented in Table 18. The degree of anisotropy in the diploic region differed significantly between HFI subtypes in males ($F=3.366$; $df=3, 15$; $p<0.05$), but the post-hoc analysis revealed no significant differences in mean values between individual groups. Other microarchitectural parameters in other regions of the frontal bone did not differ among males with different HFI types (Table 19).

Table 18. Distribution of HFI types and age in subjects who underwent micro-CT imaging of the frontal bones.

	Type A	**Type B**	**Type C**	**Type D**
Male (N)	5	6	5	3
mean age±SD (years)	53 ± 25	69 ± 10	64 ± 13	60 ± 15
Female (N)	4	4	5	4
mean age±SD (years)	80 ± 7	66 ± 26	81 ± 9	72 ± 12

Table 19. Microarchitectural parameters in males with different subtypes of HFI in different regions of frontal bone.

Microarchitectural parameter	**HFI type A (mean ± SD)**	**HFI type B (mean ± SD)**	**HFI type C (mean ± SD)**	**HFI type D (mean ± SD)**	**p value**
Total sample					
BV/TV (%)	71.55 ± 5.02	60.57 ± 9.88	67.23 ± 12.82	54.95 ± 4.48	0.096
Outer table					
BV/TV (%)	92.99 ± 5.62	92.99 ± 3.45	93.81 ± 4.69	95.32 ± 0.61	0.868
Po.Dm (mm)	0.13 ± 0.08	0.11 ± 0.04	0.11 ± 0.04	0.10 ± 0.01	0.862
Po.Tot (%)	7.01 ± 5.62	7.00 ± 3.45	6.18 ± 4.69	4.68 ± 0.61	0.868
Spongiosa					
BV/TV (%)	51.03 ± 11.97	36.06 ± 9.41	55.59 ± 19.88	40.48 ± 3.83	0.107
Tb.Th (mm)	0.27 ± 0.05	0.19 ± 0.04	0.27 ± 0.07	0.23 ± 0.03	0.056
Tb.N (1/mm)	1.91 ± 0.25	1.96 ± 0.70	2.03 ± 0.45	1.79 ± 0.29	0.917
Tb.Sp (mm)	0.50 ± 0.14	0.59 ± 0.12	0.47 ± 0.15	0.59 ± 0.14	0.496
SMI	-1.68 ± 2.07	-1.26 ± 2.29	-3.24 ± 3.31	-0.85 ± 1.07	0.499
DA	**1.97 ± 0.36**	**1.64 ± 0.26**	**2.27 ± 0.38**	**2.25 ± 0.51**	**0.047***
FD	2.57 ± 0.07	2.53 ± 0.07	2.55 ± 0.05	2.59 ± 0.02	0.610
Conn.D ($1/mm^3$)	21.27 ± 11.21	25.10 ± 10.48	14.65 ± 4.97	10.69 ± 8.01	0.136
Inner table					
BV/TV (%)	78.63 ± 10.30	69.33 ± 14.68	71.39 ± 13.81	59.16 ± 4.59	0.236
Po.Dm (mm)	0.21 ± 0.09	0.29 ± 0.15	0.28 ± 0.08	0.33 ± 0.05	0.415
Po.Tot (%)	21.37 ± 10.30	30.67 ± 14.68	28.61 ± 13.81	40.84 ± 4.59	0.236

* $p<0.050$; significant overall inter-group differences, but not between individual groups; ANOVA

Bone volume fraction increased and total porosity decreased in the outer table of males with HFI, compared to females with this condition ($t = 2.481$, $df = 34$, $p < 0.05$; $t = -2.481$; $df = 34$; $p < 0.05$, respectively). There were no significant differences regarding the investigated microarchitectural parameters in the total frontal bone sample, spongiosa and inner table (Table 20).

Table 20. Microarchitectural parameters in males and females with HFI in different regions of frontal bone.

Microarchitectural parameter	**Males with HFI (N =19) (mean ± SD)**	**Females with HFI (N =17) (mean ± SD)**	**p value**
Total sample			
BV/TV (%)	64.32 ± 10.38	56.82 ± 13.17	0.065
Outer table			
BV/TV (%)	**93.58 ± 1.00**	**89.21 ± 6.41**	**0.018***
Po.Dm (mm)	0.11 ± 0.05	0.12 ± 0.04	0.679
Po.Tot (%)	**6.42 ± 4.00**	**10.79 ± 6.41**	**0.018***
Spongiosa			
BV/TV (%)	45.84 ± 14.72	46.82 ± 16.92	0.852
Tb.Th (mm)	0.24 ± 0.06	0.22 ± 0.05	0.453
Tb.N (1/mm)	1.94 ± 0.46	2.06 ± 0.42	0.408
Tb.Sp (mm)	0.53 ± 0.13	0.48 ± 0.15	0.370
SMI	-1.83 ± 2.41	- 2.24 ± 2.79	0.634
DA	1.99 ± 0.43	1.85 ± 0.32	0.286
FD	2.55 ± 0.06	2.59 ± 0.09	0.237
Conn.D ($1/mm^3$)	19.06 ± 10.08	19.94 ± 6.60	0.763
Inner table			
BV/TV (%)	70.71 ± 12.98	72.16 ± 14.77	0.755
Po.Dm	0.28 ± 0.10	0.26 ± 0.14	0.547
Po.Tot (%)	29.29 ± 12.98	27.83 ± 12.77	0.755

* $p < 0.05$

When comparing microarchitecture of the frontal bone between males and females with corresponding types of HFI, the difference was found only regarding the fractal dimension in diploic region of HFI type C ($t = -3.431$, $df = 8$, $p < 0.05$; Tables 21-24).

Table 21. Microarchitectural parameters in males and females with HFI type A.

Microarchitectural parameter	Males (N = 5) (mean ± SD)	Females (N = 4) (mean ± SD)	p value
Total sample			
BV/TV (%)	71.55 ± 5.02	55.34 ± 19.06	0.187
Outer table			
BV/TV (%)	92.99 ± 5.62	87.70 ± 5.62	0.233
Po.Dm (mm)	0.13 ± 0.08	0.13 ± 0.04	0.973
Po.Tot (%)	7.01 ± 5.62	12.30 ± 6.25	0.223
Spongiosa			
BV/TV (%)	51.03 ± 11.97	42.40 ±15.37	0.373
Tb.Th (mm)	0.27 ± 0.05	0.20 ± 0.04	0.073
Tb.N (1/mm)	1.91 ± 0.25	2.04 ± 0.37	0.567
Tb.Sp (mm)	0.50 ± 0.14	0.50 ± 0.16	0.977
SMI	-1.68 ± 2.07	-1.50 ± 1.26	0.881
DA	1.97 ± 0.36	1.67 ± 0.07	0.136
FD	2.57 ± 0.07	2.62 ± 0.05	0.276
Conn.D ($1/mm^3$)	21.27 ± 11.21	18.74 ± 5.06	0.691
Inner table			
BV/TV (%)	78.63 ± 10.30	81.58 ± 4.58	0.223
Po.Dm (mm)	0.24 ± 0.09	0.17 ± 0.05	0.973
Po.Tot (%)	21.37 ± 10.30	18.41 ± 4.58	0.223

Table 22. Microarchitectural parameters in males and females with HFI type B.

Microarchitectural parameter	Males (N = 6) (mean ± SD)	Females (N = 4) (mean ± SD)	p value
Total sample			
BV/TV (%)	60.57 ± 9.88	50.89 ± 6.41	0.125
Outer table			
BV/TV (%)	92.99 ± 3.45	91.10 ± 5.51	0.517
Po.Dm (mm)	0.11 ± 0.04	0.11 ± 0.04	1.000
Po.Tot (%)	7.00 ± 3.45	8.00 ± 5.51	0.517
Spongiosa			
BV/TV (%)	36.06 ± 9.41	37.13 ±10.67	0.872
Tb.Th (mm)	0.19 ± 0.04	0.22 ± 0.06	0.285
Tb.N (1/mm)	1.96 ± 0.70	1.68 ± 0.50	0.506
Tb.Sp (mm)	0.59 ± 0.12	0.62 ± 0.11	0.716
SMI	-1.26 ± 2.29	-0.65 ± 1.65	0.663
DA	1.64 ± 0.26	1.95 ± 0.52	0.247
FD	2.53 ± 0.07	2.51 ± 0.13	0.754
Conn.D ($1/mm^3$)	25.10 ± 10.48	21.73 ± 5.66	0.576
Inner table			
BV/TV (%)	69.33 ± 14.68	78.00 ± 17.69	0.424
Po.Dm (mm)	0.29 ± 0.15	0.23 ± 0.17	0.566
Po.Tot (%)	30.67 ± 14.68	22.04 ± 17.69	0.424

Table 23. Microarchitectural parameters in males and females with HFI type C.

Microarchitectural parameter	Males (N = 5) (mean ± SD)	Females (N = 5) (mean ± SD)	p value
Total sample			
BV/TV (%)	67.23 ± 12.82	56.68 ± 9.35	0.168
Outer table			
BV/TV (%)	93.81 ± 4.69	87.39 ± 4.60	0.060
Po.Dm (mm)	0.11 ± 0.04	0.14 ± 0.03	0.174
Po.Tot (%)	6.18 ± 4.69	12.61 ± 4.60	0.060
Spongiosa			
BV/TV (%)	55.59 ± 19.88	52.40 ±16.30	0.788
Tb.Th (mm)	0.27 ± 0.07	0.23 ± 0.05	0.373
Tb.N (1/mm)	2.03 ± 0.45	2.21 ± 0.21	0.932
Tb.Sp (mm)	0.47 ± 0.15	0.38 ± 0.12	0.356
SMI	-3.24 ± 3.31	-2.54 ± 2.14	0.703
DA	2.27 ± 0.38	1.98 ± 0.30	0.214
FD	2.55 ± 0.05	2.64 ± 0.03	0.009*
Conn.D ($1/mm^3$)	14.65 ± 4.97	17.35 ± 5.99	0.461
Inner table			
BV/TV (%)	71.39 ± 13.81	61.30 ± 16.23	0.321
Po.Dm (mm)	0.28 ± 0.08	0.36 ± 0.13	0.278
Po.Tot (%)	28.61 ± 13.81	38.70 ± 16.23	0.321

* $p<0.05$

Table 24. Microarchitectural parameters in males and females with HFI type D.

Microarchitectural parameter	Males (N = 3) (mean ± SD)	Females (N = 4) (mean ± SD)	p value
Total sample			
BV/TV (%)	54.95 ± 4.48	64.69 ± 16.46	0.374
Outer table			
BV/TV (%)	95.32 ± 0.61	91.11 ± 10.17	0.516
Po.Dm (mm)	0.10 ± 0.01	0.09 ± 0.04	0.794
Po.Tot (%)	4.68 ± 0.61	8.89 ± 10.17	0.516
Spongiosa			
BV/TV (%)	40.48 ± 3.83	54.00 ±23.48	0.335
Tb.Th (mm)	0.23 ± 0.03	0.23 ± 0.07	0.974
Tb.N (1/mm)	1.79 ± 0.29	2.29 ± 0.46	0.163
Tb.Sp (mm)	0.59 ± 0.14	0.47 ± 0.13	0.407
SMI	-0.85 ± 1.07	-4.21 ± 4.64	0.284
DA	2.25 ± 0.51	1.77 ± 0.26	0.163
FD	2.59 ± 0.02	2.56 ± 0.08	0.623
Conn.D ($1/mm^3$)	10.69 ± 8.01	22.60 ± 10.01	0.153
Inner table			
BV/TV (%)	59.16 ± 4.59	70.53 ± 10.75	0.152
Po.Dm (mm)	0.33 ± 0.05	0.25 ± 0.12	0.321
Po.Tot (%)	40.84 ± 4.59	29.47 ± 10.75	0.152

4.2.4.1 Microarchitectural differences of the femoral bone

Males with HFI have increased bone volume fraction of the trabecular bone ($t = 2.084$, $df=36$, $p<0.05$) and decreased trabecular separation ($t = -2.279$, $df=36$, $p<0.05$) compared to males who do not have this condition (Figure 4). There are no differences in microarchitecture of cortical bone between these groups (Table 25).

Table 25. Femoral microarchitectural parameters in males with and without HFI.

Microarchitectural parameter	Males with HFI (mean ± SD)	Males without HFI (mean ± SD)	p value
Trabecular bone			
BV/TV (%)	**19.13 ± 4.74**	**16.21 ± 3.84**	**0.044***
Tb.Th (mm)	0.19 ± 0.03	0.17 ± 0.03	0.083
Tb.N (1/mm)	0.98 ± 0.17	0.93 ± 0.19	0.320
Tb.Sp (mm)	**0.87 ± 0.11**	**0.95 ± 0.11**	**0.029***
SMI	0.78 ± 0.69	1.06 ± 0.51	0.164
DA	2.48 ± 0.36	2.44 ± 0.32	0.680
FD	2.41 ± 0.07	2.37 ± 0.07	0.068
Conn.D ($1/mm^3$)	6.46 ± 3.37	6.98 ± 3.89	0.662
Cortical bone			
BV/TV (%)	74.72 ± 11.62	75.88 ± 9.88	0.742
Po.Dm (mm)	0.32 ± 0.17	0.33 ± 0.13	0.898
Po.Sp (mm)	0.28 ± 0.04	0.29 ± 0.04	0.199
Po.Cl (%)	0.19 ± 0.12	0.26 ± 0.12	0.094
Po.Op (%)	25.12 ± 11.71	23.91 ± 9.95	0.734
Po.Tot (%)	25.28 ± 11.62	24.12 ± 9.88	0.742

* $p<0.05$

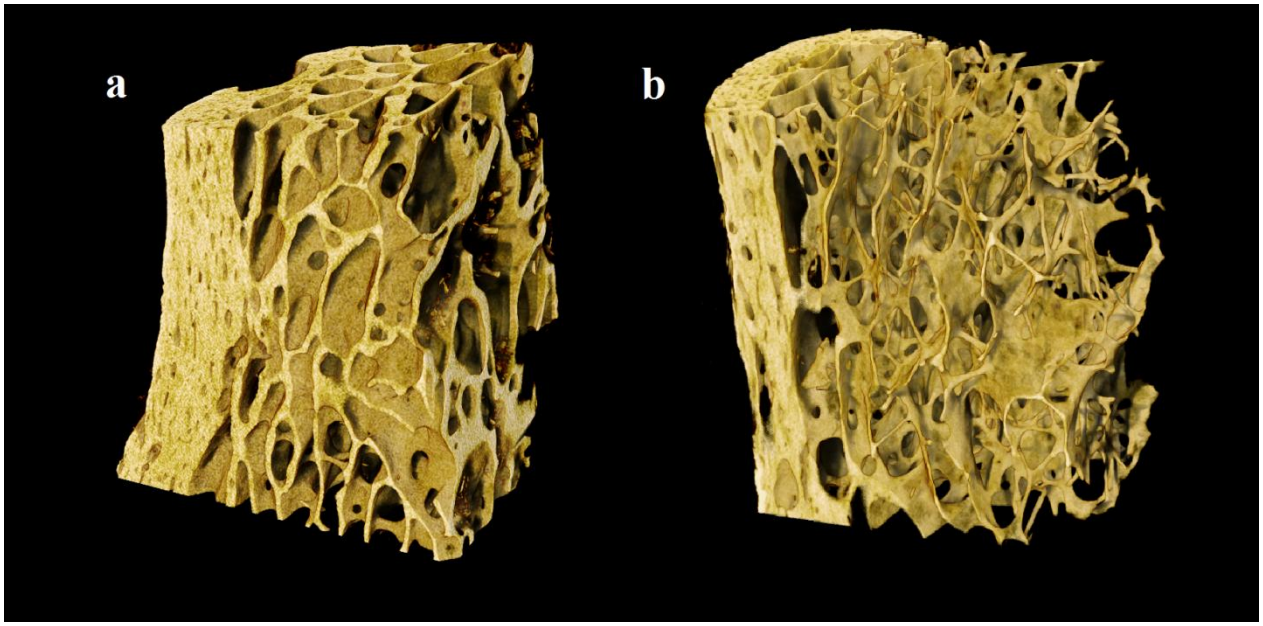

Figure 4. Micro-CT image of the femoral neck sample (lateral view);
a – 63-years-old male with HFI; **b** – 67-years-old male without HFI.

On the other hand, we detected microarchitectural changes of the cortical bone in females. Those with HFI had increased bone volume fraction ($t = 2.223$, $df=32$, $p<0.05$), increased closed porosity ($t = 2.138$, $df=32$, $p<0.05$), decreased open porosity ($t = -2.239$, $df=32$, $p<0.05$) and decreased total porosity ($t = -2.223$, $df=32$, $p<0.05$) of the cortical bone, compared to females without HFI (Figure 5). Regardless of HFI presence, the microarchitecture of the trabecular bone remained unchanged among females (Table 26).

Table 26. Femoral microarchitectural parameters in females with and without HFI.

Microarchitectural parameter (mean ± SD)	**Females with HFI (N =17)**	**Females without HFI (N =17)**	**p value**
Trabecular bone			
BV/TV (%)	16.05 ± 5.70	15.48 ± 5.04	0.760
Tb.Th (mm)	0.18 ± 0.04	0.18 ± 0.04	0.955
Tb.N (1/mm)	0.88 ± 0.25	0.84 ± 0.16	0.611
Tb.Sp (mm)	0.88 ± 0.15	0.92 ± 0.16	0.476
SMI	1.21 ± 1.22	1.46 ± 0.75	0.345
DA	2.19 ± 0.33	2.43 ± 0.44	0.100
FD	2.36 ± 0.09	2.35 ± 0.09	0.930
Conn.D ($1/mm^3$)	15.81 ± 5.05	8.28 ± 6.13	0.197
Cortical bone			
BV/TV (%)	**79.22 ± 9.52**	**72.82 ± 7.02**	**0.033***
Po.Dm (mm)	0.27 ± 0.11	0.31 ± 0.08	0.260
Po.Sp (mm)	0.30 ± 0.04	0.30 ± 0.05	0.808
Po.Cl (%)	**0.24 ± 0.14**	**0.16 ± 0.09**	**0.040***
Po.Op (%)	**20.57 ± 9.63**	**27.06 ± 7.07**	**0.032***
Po.Tot (%)	**20.76 ± 9.52**	**27.18 ± 7.02**	**0.040***
SD.PoDm	0.19 ± 0.07	0.20 ± 0.07	0.459
SD.PoSp	0.09 ± 0.01	0.09 ± 0.01	0.861

* $p<0.05$

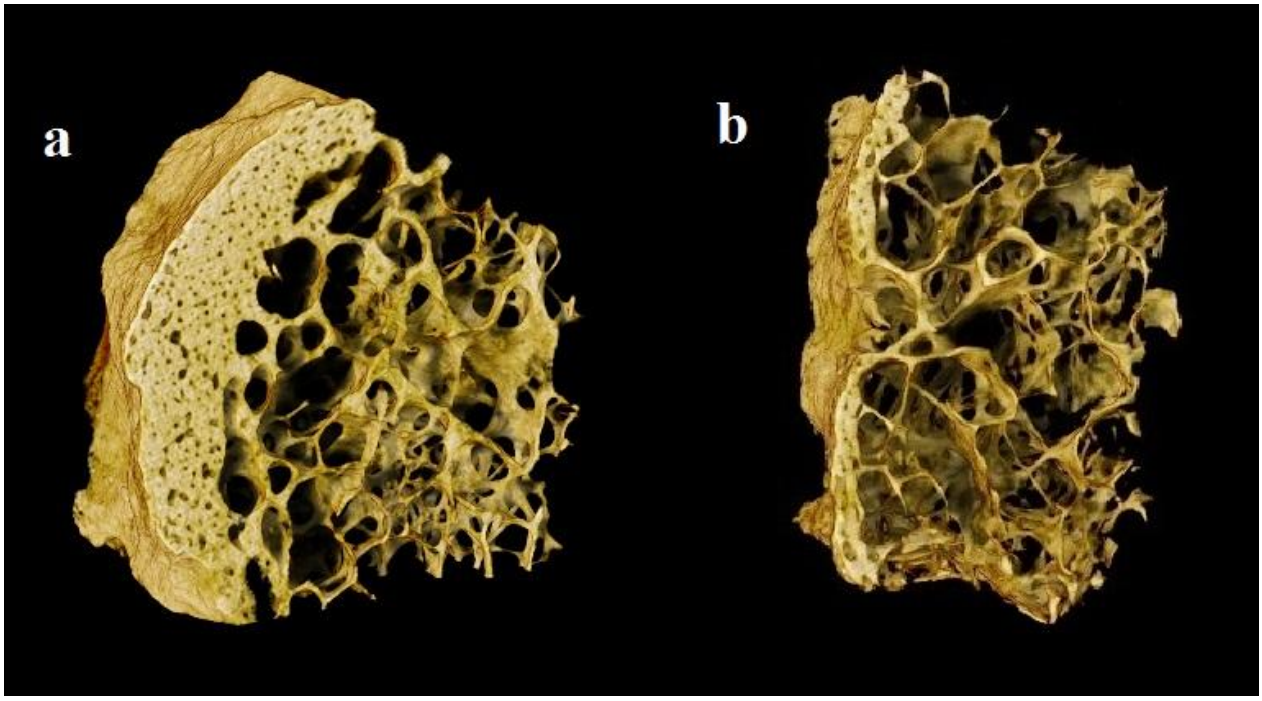

Figure 5. Micro-CT image of the femoral neck sample (view from above);
a – 86-years-old female with HFI; **b** – 86-years-old female without HFI.

When microarchitectural parameters of the trabecular bone in males and females with HFI were compared, statistical differences were detected in unadjusted data, regarding the degree of anisotropy of the trabecular bone (t= 2.466, df=34, p<0.05). Borderline significance was reached for structure model index (p=0.080), fractal dimension (p=0.055) and connectivity density (p=0.055; Table 27). However, after adjusting data for age, height and weight, the degree of anisotropy only slightly increased among males with HFI (F=3.961, df=1, p=0.055), while other differences disappeared (Table 27), suggesting similar bone microarchitecture in all subjects with HFI, male and female.

There were no differences regarding the microarchitectural parameters of the cortical bone of proximal femora between males and females with HFI (Table 28).

Table 27. Microarchitectural parameters of trabecular bone in males and females with HFI, before and after adjustment for age, height and weight (covariates appearing in the corrected model are evaluated at the following values: age=67.47 years; height=171.44cm; weight=79.06 kg).

Microarchitectural parameter	**Males with HFI (mean ± SD)**	**Females with HFI (mean ± SD)**		**p value**
N	19	17		
Before adjustment				
BV/TV (%)	19.13 ± 4.74	16.05 ± 5.70		0.086
Tb.Th (mm)	0.19 ± 0.03	0.18 ± 0.04		0.401
Tb.N (1/mm)	0.98 ± 0.17	0.88 ± 0.25		0.140
Tb.Sp (mm)	0.87 ± 0.11	0.88 ± 0.15		0.745
SMI	0.78 ± 0.69	1.21 ± 1.22		**0.080**
DA	2.48 ± 0.36	2.19 ± 0.33		**0.019***
FD	2.41 ± 0.07	2.36 ± 0.09		**0.055**
Conn.D ($1/mm^3$)	6.46 ± 3.37	15.81 ± 5.05		**0.055**
After adjustment	**(mean± SE)**	**(mean± SE)**	**Corrected model**	
BV/TV (%)	18.45 ± 1.47	16.82 ± 1.60	0.019*	0.539
Tb.Th (mm)	0.20 ± 0.01	0.18 ± 0.01	0.293	0.371
Tb.N (1/mm)	0.93 ± 0.06	0.95 ± 0.07	0.022*	0.884
Tb.Sp (mm)	0.89 ± 0.04	0.86 ± 0.04	0.317	0.689
SMI	0.91 ± 0.21	1.07 ± 0.24	0.076	**0.684**
DA	2.52 ± 0.10	2.16 ± 0.11	**0.029***	**0.055**
FD	2.39 ± 0.02	2.38 ± 0.03	**0.031***	**0.768**
Conn.D ($1/mm^3$)	3.65 ± 5.01	19.00 ± 5.43	**0.345**	**0.097**

* p<0.05

Table 28. Microarchitectural parameters of cortical bone in males and females with HFI, before and after adjustment for age, height and weight (covariates appearing in the corrected model are evaluated at the following values: age=67.47 years; height=171.44cm; weight=79.06 kg).

Microarchitectural parameter	**Males with HFI (mean± SE)**	**Females with HFI (mean ± SE)**		**p value**
N	19	17		
Before adjustment				
BV/TV (%)	74.72 ± 11.62	79.22 ± 9.52		0.215
Po.Dm (mm)	0.32 ± 0.17	0.27 ± 0.11		0.284
Po.Sp (mm)	0.28 ± 0.04	0.30 ± 0.04		0.141
Po.Cl (%)	0.19 ± 0.12	0.24 ± 0.14		0.302
Po.Op (%)	25.12 ± 11.71	20.57 ± 9.63		0.215
Po.Tot (%)	25.28 ± 11.62	20.76 ± 9.52		0.215
After adjustment	**(mean± SE)**	**(mean± SE)**	**Corrected Model**	
BV/TV (%)	76.64 ± 3.42	77.08 ± 3.71	0.663	0.945
Po.Dm (mm)	0.35 ± 0.05	0.24 ± 0.05	0.675	0.184
Po.Sp (mm)	0.30 ± 0.01	0.28 ± 0.01	0.045*	0.343
Po.Cl (%)	0.20 ± 0.04	0.25 ± 0.05	0.829	0.536
Po.Op (%)	23.20 ± 3.45	22.72 ± 3.75	0.664	0.938
Po.Tot (%)	23.36 ± 3.42	22.92 ± 3.71	0.663	0.943

* $p<0.05$

4.3. Comparative analysis of femoral macro and micromorphology

One of the challenges we encountered during this research was to make a precise distinction between milder and severe forms of HFI observed during autopsy. For that reason, having done the initial analysis, we reclassified the group with HFI into two groups: milder HFI (comprising original types A and B) and severe HFI (types C and D). We then compared femoral macro and micromorphology of these two groups with the femora of subjects who did not have HFI (control group).

4.3.1 *Densitometric measurements*

In both sexes, densitometric measurements did not show any statistically significant differences between these three groups, neither before nor after adjustment for height and height (Tables 29 and 30). However, it could be noticed that in males mean value of bone mineral content and density in both observed regions (neck and total femoral sample) tend to increase with the occurrence of a more severe form of HFI. Spearman's Rho for the correlation between the total bone mineral density and the severity of HFI in males is 0.303, and the correlation reached borderline statistical significance (p=0.065). In females, there was no correlation between any of the DXA parameters and HFI severity.

Table 29. DXA analysis of males without HFI, males with milder (A and B) and severe (C and D) HFI subtypes, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=177.68 cm; weight=87.26 kg).

DXA parameter before adjusting	**Males without HFI (Mean ± SD)**	**Males with HFI A and B (Mean ± SD)**	**Males with HFI C and D (Mean ± SD)**		**p value**
N	19	12	7		
BA n (cm^2)	5.41 ± 0.92	5.71 ± 0.87	5.77 ± 0.85		0.536
BA t (cm^2)	53.41 ± 8.97	51.88 ± 5.70	56.27 ± 7.93		0.512
BMC n (g)	4.14 ± 1.04	4.54 ± 1.34	4.91 ± 1.22		0.304
BMC t (g)	50.72 ± 9.44	52.32 ± 11.38	62.27 ± 20.96		0.130
BMD n (g/cm^2)	0.76 ± 0.11	0.78 ± 0.18	0.86 ± 0.22		0.335
BMD t (g/cm^2)	0.95 ± 0.13	1.00 ± 0.18	1.09 ± 0.26		0.220
DXA parameter after adjusting	**(Mean ± SE)**	**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
BA n (cm^2)	5.41 ± 0.21	5.68 ± 0.26	5.80 ± 0.33	0.369	0.551
BA t (cm^2)	54.21 ± 1.19	50.81 ± 2.25	55.90 ± 2.89	0.204	0.333
BMC n (g)	4.20 ± 0.27	4.45 ± 0.34	4.91 ± 0.44	0.277	0.406
BMC t (g)	52.00 ± 2.84	50.53 ± 3.56	61.84 ± 4.58	0.047*	0.130
BMD n (g/cm^2)	0.77 ± 0.04	0.76 ± 0.05	0.86 ± 0.06	0.390	0.449
BMD t (g/cm^2)	0.97 ± 0.04	0.99 ± 0.05	1.09 ± 0.07	0.292	0.309

n – femoral neck region; *t* – total femoral sample

*$p<0.05$

Table 30. DXA analysis of females without HFI, females with milder (A and B) and severe (C and D) HFI subtypes, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=161.85 cm; weight=62.56 kg).

DXA parameter before adjusting	**Females without HFI (Mean ± SD)**	**Females with HFI A and B (Mean ± SD)**	**Females with HFI C and D (Mean ± SD)**		**p value**
N	17	7	10		
BA n (cm^2)	4.98 ± 1.08	4.26 ± 1.48	4.74 ± 0.91		0.380
BA t (cm^2)	49.16 ± 6.52	50.86 ± 5.77	45.95 ± 3.98		0.202
BMC n (g)	3.10 ± 1.16	2.53 ± 0.77	2.91 ± 1.04		0.496
BMC t (g)	40.29 ± 10.80	40.92 ± 6.63	37.81 ± 10.68		0.776
BMD n (g/cm^2)	0.62 ± 0.17	0.63 ± 0.19	0.61 ± 0.13		0.960
BMD t (g/cm^2)	0.81 ± 0.16	0.82 ± 0.19	0.81 ± 0.17		0.999
DXA parameter after adjusting	**(Mean ± SE)**	**(Mean ± SE)**	**(Mean ± SE)**	**Corrected model**	
BA n (cm^2)	4.97 ± 0.28	4.20 ± 0.44	4.79 ± 0.38	0.565	0.336
BA t (cm^2)	49.21 ± 1.45	51.15 ± 2.25	45.68 ± 1.97	0.437	0.197
BMC n (g)	3.15 ± 0.26	2.65 ± 0.40	2.74 ± 0.35	0.371	0.478
BMC t (g)	40.85 ± 2.12	42.93 ± 3.29	35.45 ± 2.88	0.016*	0.219
BMD n (g/cm^2)	0.63 ± 0.03	0.66 ± 0.05	0.56 ± 0.05	0.024*	0.380
BMD t (g/cm^2)	0.83 ± 0.04	0.85 ± 0.05	0.77 ± 0.05	0.008*	0.470

n – femoral neck region; *t* – total femoral sample

*p<0.05

4.3.2 ***Hip structure analysis***

Cross-sectional area of males in the control group increased in all regions compared to both groups with HFI, and this difference remained significant in the neck area even after adjustment (F=54.514, df=2, p<0.05; Table 31). In females, the control group also had the highest CSA values in the neck region (before and after adjustment; F=49.183, df=2, p<0.05), but the lowest in the shaft region (F=8.586, df=2, p<0.05; Table 32).

Table 31. HSA in males without HFI, males with milder (A and B) and severe (C and D) HFI subtypes, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=177.68 cm; weight=87.26 kg).

HSA parameter before adjusting	Region	Males without HFI (Mean ± SD)	Males with HFI A and B (Mean ± SD)	Males with HFI C and D (Mean ± SD)		p value
N		19	12	7		
CSA (cm^2)	NN	6.61 ± 1.51 [a, b]	3.34 ± 0.81	3.75 ± 0.94		0.000*
	IT	5.06 ± 0.93	7.70 ± 2.98	8.13 ± 6.03		0.048*
	FS	3.85 ± 1.15 [a]	5.41 ± 0.78	4.07 ± 3.38		0.049*
SM (cm^3)	NN	1.90 ± 0.42	2.16 ± 0.57	2.40 ± 0.75		0.101
	IT	6.88 ± 2.22	9.048 ± 4.79	8.50 ± 6.19		0.287
	FS	3.05 ± 0.63	3.37 ± 0.35	2.65 ± 2.42		0.430
CTh (cm)	NN	0.17 ± 0.03	0.18 ± 0.04	0.20 ± 0.05		0.370
	IT	0.88 ± 0.74	0.88 ± 0.74	0.88 ± 0.74		0.318
	FS	0.79 ± 0.51	0.57 ± 0.25	0.86 ± 0.49		0.251
BR	NN	11.94 ± 2.59	12.58 ± 8.50	14.53 ± 3.13		0.708
	IT	7.75 ± 1.53	6.52 ± 3.72	10.60 ± 3.14		0.083
	FS	2.72 ± 0.60	3.36 ± 1.74	3.83 ± 0.42		0.178
HSA parameter after adjusting		**(Mean ± SE)**		**(Mean ± SE)**	**Corrected model**	
CSA (cm^2)	**NN**	**6.90 ± 0.25 [a, b]**	**3.05 ± 0.28**	**3.66 ± 0.36**	**0.000***	**0.000***
	IT	5.34 ± 0.80	7.33 ± 0.92	8.13 ± 1.17	0.035*	0.119
	FS	4.10 ± 0.36	5.10 ± 0.45	3.96 ± 0.58	0.005*	0.172
SM (cm^3)	NN	1.96 ± 0.14	2.07 ± 0.16	2.37 ± 0.20	0.030*	0.258
	IT	7.62 ± 1.01	8.68 ± 1.12	8.29 ± 1.44	0.028*	0.787
	FS	3.15 ± 0.29	3.20 ± 0.33	2.60 ± 0.42	0.153	0.486
CTh (cm)	NN	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.02	0.832	0.548
	IT	0.58 ± 0.09	0.57 ± 0.09	0.86 ± 0.11	0.300	0.100
	FS	0.80 ± 0.11	0.64 ± 0.11	0.50 ± 0.14	0.235	0.240
BR	NN	11.94 ± 1.47	12.41 ± 1.91	14.79 ± 3.23	0.780	0.730
	IT	7.81 ± 0.70	6.39 ± 0.91	10.71 ± 1.53	0.170	0.068
	FS	2.75 ± 0.30	3.35 ± 0.39	3.89 ± 0.66	0.408	0.244

* $p<0.05$

[a] Significant difference between males without HFI and males with milder HFI subtypes; ANOVA
[b] Significant difference between males without HFI and males with severe HFI subtypes; ANOVA

Table 32. HSA in females without HFI, females with milder (A and B) and severe (C and D) HFI subtypes, before and after adjustment for height and weight (covariates appearing in the corrected model are evaluated at the following values: height=161.76 cm; weight=63.55 kg).

HSA parameter before adjusting	Region	Females without HFI (Mean ± SD)	Females with HFI A and B (Mean ± SD)	Females with HFI C and D (Mean ± SD)		p value
N		17	7	10		
CSA (cm^2)	**NN**	**5.18 ± 1.24 [a,b]**	**2.41 ± 0.55**	**2.29 ± 0.50**		**0.000***
	IT	3.89 ± 0.69	4.55 ± 2.12	5.42 ± 2.34		0.079
	FS	**2.41 ± 0.75[a,b]**	**4.00 ± 0.69**	**3.60 ± 1.49**		**0.002***
SM (cm^3)	NN	1.28 ± 0.36	1.23 ± 0.23	1.17 ± 0.37		0.706
	IT	5.31 ± 1.53	5.10 ± 2.34	6.14 ± 3.07		0.564
	FS	2.28 ± 0.35	2.01 ± 0.71	2.21 ± 0.85		0.435
CTh (cm)	NN	0.14 ± 0.04	0.16 ± 0.05	0.14 ± 0.04		0.783
	IT	0.49 ± 0.27	0.37 ± 0.19	0.49 ± 0.37		0.636
	FS	0.51 ± 0.13	0.57 ± 0.25	0.43 ± 0.20		0.311
BR	NN	14.37 ± 5.71	11.64 ± 1.93	14.56 ± 4.01		0.524
	IT	9.84 ± 2.79	7.08 ± 1.63	6.97 ± 3.57		0.047
	FS	3.33 ± 1.09	4.60 ± 4.17	2.31 ± 2.23		0.150
HSA parameter after adjusting		**(Mean ± SE)**		**(Mean ± SE)**	**Corrected model**	
CSA (cm^2)	**NN**	**5.25 ± 0.21[a,b]**	**2.60 ± 0.33**	**2.06 ± 0.29**	**0.000***	**0.000***
	IT	3.93 ± 0.35	4.85 ± 0.55	5.14 ± 0.48	0.004*	0.116
	FS	**2.44 ± 0.23[a,b]**	**4.15 ± 0.36**	**3.45 ± 0.32**	**0.001***	**0.001***
SM (cm^3)	NN	1.31 ± 0.08	1.29 ± 0.12	1.09 ± 0.11	0.091	0.256
	IT	5.34 ± 0.51	5.44 ± 0.78	5.86 ± 0.69	0.072	0.836
	FS	2.28 ± 0.15	2.05 ± 0.23	2.17 ± 0.20	0.662	0.700
CTh (cm)	NN	0.15 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.042*	0.218
	IT	0.44 ± 0.07	0.39 ± 0.09	0.46 ± 0.08	0.708	0.828
	FS	0.52 ± 0.05	0.60 ± 0.06	0.40 ± 0.06	0.030*	0.081
BR	NN	14.15 ± 1.27	11.51 ± 2.22	15.18 ± 2.21	0.520	0.475
	IT	9.63 ± 0.66	6.96 ± 1.15	7.56 ± 1.15	0.010*	0.097
	FS	3.12 ± 0.45	4.48 ± 0.78	2.91 ± 0.78	0.024*	0.271

* $p<0.05$

[a] Significant difference between females without HFI and females with milder HFI subtypes; ANOVA

[b] Significant difference between females without HFI and females with severe HFI subtypes; ANOVA

4.3.3 *Microarchitectural analysis*

The structural model index of the trabecular bone differed significantly between all three male groups (F=4.364, df=2, 35, $p<0.05$; Table 33), while there were no changes in the microarchitecture of the cortical bone. In females, trabecular number and connectivity density were the highest in the group of milder HFI subtype compared to other groups (F=5.316, df=2, 31, $p<0.05$; $\chi^2=9.135$; df=3; $p<0.05$; Table 34). Pore diameter of the cortical bone differed significantly between females (F=3.768; df=2, 31; $p<0.05$), but the post-hoc analysis revealed no significant differences in mean values between the individual groups (Table 34).

Table 33. Femoral microarchitectural parameters in males without HFI, males with milder (A and B) and severe (C and D) HFI subtypes.

Microarchitectural parameter	**Males without HFI (mean ± SD)**	**Males with HFI type A and B (mean ± SD)**	**Males with HFI type C and D (mean ± SD)**	**p value**
N	19	12	7	
Trabecular bone				
BV/TV (%)	16.21 ± 3.84	19.14 ± 3.80	19.11 ± 6.40	0.136
Tb.Th (mm)	0.17 ± 0.03	0.19 ± 0.03	0.20 ± 0.03	0.219
Tb.N (1/mm)	0.93 ± 0.19	1.00 ± 0.16	1.00 ± 0.20	0.568
Tb.Sp (mm)	0.95 ± 0.11	0.88 ± 0.11	0.86 ± 0.12	0.089
SMI	**1.06 ± 0.51[a]**	**0.53 ± 0.47 [b]**	**1.21 ± 0.82**	**0.020***
DA	2.44 ± 0.32	2.51 ± 0.39	2.43 ± 0.32	0.769
FD	2.37 ± 0.07	2.41 ± 0.07	2.43 ± 0.32	0.195
Conn.D ($1/mm^3$)	6.98 ± 3.89	5.91 ± 3.03	7.42 ± 3.94	0.625
Cortical bone				
BV/TV (%)	75.88 ± 9.88	72.62 ± 11.50	78.33 ± 11.79	0.514
Po.Dm (mm)	0.33 ± 0.13	0.35 ± 0.19	0.28 ± 0.11	0.639
Po.Sp (mm)	0.29 ± 0.04	0.27 ± 0.04	0.27 ± 0.04	0.236
Po.Cl (%)	0.26 ± 0.11	0.17 ± 0.11	0.24 ± 0.14	0.089
Po.Op (%)	23.91 ± 9.95	27.37 ± 11.57	21.47 ± 11.89	0.509
Po.Tot (%)	24.12 ± 9.88	27.38 ± 11.50	21.68 ± 11.79	0.514

* $p<0.05$

[a] Significant difference between males without HFI and males with milder HFI subtypes; ANOVA
[b] Significant difference between the males with milder and severe HFI subtypes; ANOVA

Table 34. Femoral microarchitectural parameters in females without HFI, females with milder (A and B) and severe (C and D) HFI subtypes.

Microarchitectural parameter	Females without HFI (mean ± SD)	Females with HFI type A and B (mean ± SD)	Females with HFI type C and D (mean ± SD)	p value
N	17	7	10	
Trabecular bone				
BV/TV (%)	15.48 ± 5.04	18.41 ± 5.78	14.40 ± 5.31	0.303
Tb.Th (mm)	0.18 ± 0.04	0.18 ± 0.43	0.19 ± 0.04	0.888
Tb.N (1/mm)	**0.84 ± 0.16** [a]	**1.05 ± 0.28** [b]	**0.76 ± 0.14**	**0.010***
Tb.Sp (mm)	0.92 ± 0.16	0.78 ± 0.09	0.96 ± 0.15	0.054
SMI	1.46 ± 0.75	1.40 ± 1.07	1.10 ± 0.43	0.478
DA	2.43 ± 0.44	2.08 ± 0.34	2.28 ± 0.32	0.156
FD	2.35 ± 0.09	2.43 ± 0.08	2.32 ± 0.08	0.054
Conn.D (1/mm^3)	**8.28 ± 6.13** [c]	**28.39 ± 32.24** [d]	**7.00 ± 4.50**	**0.011***
Cortical bone				
BV/TV (%)	72.82 ± 7.02	78.93 ± 11.00	79.42 ± 9.00	0.105
Po.Dm (mm)	**0.31 ± 0.08**	**0.33 ± 0.12**	**0.23 ± 0.08**	**0.034***
Po.Sp (mm)	0.30 ± 0.05	0.33 ± 0.04	0.28 ± 0.03	0.181
Po.Cl (%)	0.16 ± 0.09	0.27 ± 0.16	0.22 ± 0.13	0.087
Po.Op (%)	27.06 ± 7.07	20.83 ± 11.07	20.39 ± 9.12	0.104
Po.Tot (%)	27.18 ± 7.02	21.06 ± 10.95	20.57 ± 9.00	0.105

* $p<0.05$

[a] Significant difference between females without HFI and females with milder HFI subtypes; ANOVA
[b] Significant difference between females with milder and severe HFI subtypes; ANOVA
[c] Significant difference between females without HFI and females with milder HFI subtypes; Kruskal-Wallis test
[d] Significant difference between females with milder and severe HFI subtypes; Kruskal-Wallis test

Spearman's correlation was done for microarchitectural parameters of the trabecular and cortical bone in the three previously defined groups. In males, negative correlation was found between trabecular separation and the presence/severity of HFI (Table 35). Some correlations reached borderline significance: in females, there was positive correlation between the presence/severity of HFI and cortical bone volume fraction and negative for open and total porosity (Table 36).

Trabecular thickness in females negatively correlated with age (Spearman's Rho= – 0.352, $p<0.05$). All the other instigated microarchitectural parameters did not correlate with age in either sex.

Table 35. Correlation between the presence/severity of HFI (without HFI, milder, severe) and microarchitectural parameters of the trabecular bone.

	BV/TV	Tb.Th	Tb.N	Tb.Sp	SMI	DA	FD	Conn.D
Males (N=38)								
Spearman's Rho	0.273	0.206	0.140	**-0.351**	-0.179	0.032	0.280	-0.001
p value	0.098	0.214	0.401	**0.031***	0.283	0.849	0.089	0.997
Females (N=34)								
Spearman's Rho	-0.056	0.006	-0.126	-0.077	-0.121	-0.125	-0.116	0.028
p value	0.763	0.972	0.479	0.665	0.502	0.480	0.522	0.876

* $p<0.05$

Table 36. Correlation between the presence/severity of HFI (without HFI, milder, severe) and microarchitectural parameters of the cortical bone.

	BV/TV	Po.Dm	Po.Sp	Po.Cl	Po.Op	Po.Tot
Males (N=38)						
Spearman's Rho	0.014	-0.092	-0.139	-0.248	-0.024	-0.014
p value	0.931	0.583	0.406	0.133	0.886	0.931
Females (N=34)						
Spearman's Rho	**0.331**	-0.312	-0.016	-0.279	**-0.335**	**-0.331**
p value	**0.056**	0.072	0.928	0.110	**0.053**	**0.056**

* $p<0.05$

5 Discussion

Among the studies focused on HFI as the subject of research and whose study sample included modern autopsy material, to our knowledge this research comprised the largest cadaveric sample of males with HFI (41 males). Herein, we confirmed that HFI is not a purely a female phenomenon. It seems that the frequency, some types of local and systemic magnitude of manifestation of this condition is different among males and females.

In our study sample, logistic regression has shown that age is a predictor of HFI occurrence in females, but not in males. Males had the same risk of HFI occurrence as females below 55 years of age, but after this age HFI manifestation starts to be age-related only in females. In our study sample, males with HFI were younger than females with HFI. The peak incidence for HFI occurrence in males was in the 61–70 age group, and after that it gradually declined. In females, however, the incidence was progressively increasing with age. Similar results were obtained in other research [7,13,18,51]. Hershkovitz et al. noted that HFI was much less frequent in females under 40 years of age and seemed to be age-dependent, increasing from 11.8% in the youngest female age group (20–29 years) to 44.2% in the oldest (aged 80 and over) [7]. Western et al. conducted a research on the skulls of females living in the industrial period (from 17^{th} untill 19^{th} century) and concluded that HFI prevalence was expected to be increasing up to 50–59 years age group. Following that, HFI rates appeared to remain relatively consistent. They considered the age above 50 to be “the plateau” for HFI occurrence, at least in females [8]. This could not be supported by our findings. However, there is a great difference between the sizes of the two study samples (out of 138 females, only 22 had HFI in their study). May et al. made an observation that the development of HFI in a healthy male was more probably caused by genetics and congenital factors rather than age [48]. Most of the original studies on HFI were carried out on a disproportionately small male sample (or in some instances, only on female population), which is why we cannot compare our observations about age and HFI occurrence in males.

Our results indicated that females younger than 55 years of age had the same risk of HFI occurrence as males, while in women aged 55 and older HFI manifestation started to be age-related. It seems that the critical event that makes the difference between these two groups is the menopause, which causes a shift in hormonal levels which probably influences HFI pathogenesis. The menopause refers to a point in time that follows 1 year after the complete cessation of menstruation, and the postmenopause describes the years following that point. The average age of its onset is 47, and menopausal transition typically spans from 4 to 7 years [70]. As many researchers proposed, it seems that a sudden decrease in estrogen production could be the trigger for HFI occurrence or its transition into more severe forms. As histological and micro-CT studies have shown, this process is still rather slow [7,12,24]. Based on our results, we can predict (with a sensitivity of 73% and specificity of 63%) that approximately 13.5 years following menopause (around 69 years of age) HFI could occur in females. This theory could explain why HFI is more common in females (compared to males) and why older females have more severe forms of HFI. In males and females under 55, some other factors could influence the occurrence of HFI, and available literature data [7,8,17,42] indicate that those factors are probably also related to hormonal disturbances.

We agree with the statement of Hershkovitz that, instead of asking whether the relative preponderance of HFI in females is a clue to its etiology, the question is whether the low frequency and intensity of HFI among males can give us some answers regarding its etiology [7]. Does testosterone suppression [17] or elevation [8] play the pivotal role or does maybe abnormal estrogen surplus lead to bone formation typical of HFI? In that sense, it could be an inherent condition or a condition acquired in early adulthood that leads to relative or absolute hypogonadism (Klinefelter's syndrome [55], testicular atrophy [7], obesity [71]), or some other factor that most probably does not have the potency to cause severe forms of HFI. Sex hormone levels are positively correlated with each other as a result of all being part of the same metabolic pathway [72] and, at a bottom line, the altered sex hormone ratio might be the most probable etiological factor. However, only prospective clinical controlled studies, with the measurements of hormone levels and consecutive head CT scans, could confirm this theory, but such a study would be very hard to conduct.

In males, estrogen regulates bone growth, glucose and lipid metabolism and gonadotropin levels [73–75]. Testes produce only 15–20% of circulating estrogens and the remainder comes from local production by adipose tissue, brain, skin and bone, where testosterone is converted to estrogen through aromatase actions [76,77]. Testosterone concentrations in males are at least two orders of magnitude greater than estrogen concentrations and this may vary with age, higher concentrations prepubertally and gradual age-related decline [78]. The most potent estrogen is estradiol (E2). In males, E2 production requires aromatization with ubiquitous cytochrome P450 reductase enzyme [79]. Estrogens are inactivated through sulfoconjugation, which is catalyzed by estrogen sulfotransferase that is abundantly expressed in liver and other organs [80]. This implies that the concentration or the level of activity of this enzyme can also affect estrogen concentrations in males. Increase in serum E2 and decreased testosterone/E2 ratio in elderly males could be the result of age-related decreased expression of estrogen sulfotransferase and decreases in testosterone production [73,76]. If we considered estrogen surplus or testosterone decline in elderly males *the only* causes of HFI occurrence, it would be expected that the frequency of HFI increases with ageing. According to the results of our study this was not the case – HFI occurrence in males was not related to age (unlike in females) and older subjects did not have more severe forms of HFI. In our opinion, there are some other factors (probably still unknown) which act in synergism with hormonal disturbances, leading to HFI occurrence in males.

Natural cases of excessive aromatase activity (EAA) causing estrogen excess in males have been reported. The condition is transmitted as an autosomal dominant trait [81,82]. These males have normal male sexual differentiation, pre- or peripubertal gynecomastia, micro-orchidism, accelerated prepubertal growth, advanced bone age and tall childhood stature. EAA adults exhibit normal to slightly reduced height and hypogonadotropic hypogonadism with low to normal testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and normal to high serum E2 [83]. Serum E2/testosterone ratios are also elevated [84].

Compared to females, HFI in males is usually moderate in extent, usually of types A and B, while type C and D are rarely reported [7,13,18]. Many agree that this could be the reason for underestimation of HFI prevalence in males, especially in radiological studies where a small isolated bony island (typical for type A) could be overlooked due to superimposition or inexperience [11]. Also, if the pathologist does not carefully inspect the skull-cap (after meticulous removal of the dura), they could easily fail to observe these small lesions at the autopsy.

The presence of less severe forms of HFI was also the most frequent in our male sample (combined, type A and B comprised 66%). However, we recorded as much as 4 out of 41 males (10%) with the most severe, type D HFI. These subjects were very heterogenic in the terms of age (44, 63, 65 and 79 years) which is in accordance with the statistical analysis that showed that the severity of HFI did not correlate with age. That high proportion of males with the most severe form of HFI is quite a rare finding, since in the entire male skeletal sample (comprising 3725 historic and modern skulls), Hershkovitz et al. reported only one case of HFI type C and no cases of type D, and only one of each of those types in the modern cadaveric sample (72 skulls). In their study, the majority of cases were type A: 60% among European American males (most of them were over 50) and 76% among African American males (most were under 50 years of age) [7]. Since type B was the most common type among males in our study (46%) and the peak incidence was in the 61–70 age group, the reason might be linked to the fact that our study sample was "more modern" and therefore prone to different lifestyles (e.g. diet more contaminated with hormones, the use of steroids for physical enhancement, etc.). Like other researchers, we also firmly believe that males probably developed HFI type D under extreme conditions of hormonal imbalance [7,17,55].

In our study, the severity of HFI did not correlate with age either in males or females. A relationship between HFI severity and the patient's age was noted in both males and females suffering from acromegaly [37]. In some studies, the magnitude of HFI manifestation in females increased with age [7,38], while in others this correlation has not been proved [18]. Once again, the conclusions regarding correlation between HFI severity and age of males are lacking in literature. May et al. have shown the positive association (albeit without statistical significance) between HFI prevalence (not severity) and the duration of hormonal androgen block treatment in males with prostate

cancer [17]. Maybe our results could point out that, at least in males, HFI severity correlates better with the intensity of potential etiological factor rather than the exposure duration.

Many considered the possibility that any etiological factor contributing to HFI could also affect other skull bones as well. The first classification of calvarial hyperostosis that Moore introduced was primarily based on the observation that bone formation can appear on the inner surface of a different (or any) skull bone. As we have already mentioned, he considered HFI (hyperostosis located on the frontal bone) and HCD (diffuse hyperostosis of the calvaria) to be different, gradual manifestations of the same process [7]. On the other hand, Perou did not consider HCD a condition per se, but rather the end result of several different unrelated pathological processes [6]. Hershkovitz argued that HFI and HCD should be considered as separate conditions with different etiology, which could still occur at the same time. In their study, the coexistence of HFI and HCD was not related to the extent of HFI (of skulls with HCD, 17.6% were associated with HFI type A and 19.5% were associated with HFI type D) [7].

In our sample, all subjects with HFI (male and female) had thicker frontal and temporal bones, which is in accordance with the results of the previous studies conducted on females [14,18,38]. Furthermore, the thickness of the frontal, temporal and occipital bones was positively correlated with the severity of HFI in males. As expected, the correlation was the strongest for the frontal bone (in both sexes) – the higher the grade of HFI, the thicker the frontal bone. Since the thickening of the inner table is rarely seen only on the frontal bone, it is still debatable whether HFI and HCD are different manifestations of the same process. We proved that other skull bones are thicker in subjects with HFI; however, that thickening does not resemble the morphology typical of HFI. In our opinion, HFI is the condition that primary affects the frontal bone, but if there is a local etiological factor that leads to its formation, it probably affects other skull bones as well, yet to a lesser extent. That factor could be a tissue growth factor with paracrine effect or some other still unidentified factor(s) that may have either regional or systemic effect on other skeletal sites.

We also wanted to test the hypothesis that the process of HFI development influences the cranial vault volume; therefore, besides the thickness of the skull bones, we measured longitudinal and frontal diameters of the skulls. Association between HFI and neurological disorders has not been proven; however, HFI is frequently described together with neuropsychiatric symptoms including frontal executive dysfunctions, dementia, depression, epilepsy, cognitive impairments, parkinsonism and frontal headache [59,85–87]. May et al. even suggested that the presence of HFI could imply a decrease in brain volume which may indicate the beginning of degenerative processes of the brain [38]. As our results have shown, females with HFI do have slightly smaller longitudinal diameter compared to females without HFI, which is probably due to the thicker frontal and occipital bones. Since HFI occurrence in females is age-related, it is difficult to say whether neuropsychiatric symptoms are the consequence of this condition or of ageing per se. The most severe cases of HFI occur in elderly females who usually have concomitant brain atrophy. In our opinion, in those females smaller cranial vault size is "compensated" by smaller brain size. Both HFI and degenerative brain atrophy are slow progressive conditions which could be the reason why HFI is frequently merely an accidental radiological finding, still considered clinically irrelevant. Males, on the other hand, did not show differences in skull diameters. They also have milder HFI types which do not cause any symptoms and are usually discovered only at the autopsy.

Before the invention of microcomputed tomography (micro-CT), histological examination was the only method for evaluation of microarchitectural parameters of bones, such as trabecular thickness. Histopathology still represents an irreplaceable method for the diagnosis of bone pathologies in clinical medicine [88], but micro-CT imaging allows insight into the three-dimensional microstructure of the bone and, therefore, opens discussions regarding the pathogenesis of different bone conditions.

Microarchitectural analysis of the skull bone with no pathology clearly shows classic three layers, with the outer table consisting of compact bone that is nearly 1 mm thick. The spongiosa is built of cancellous bone with plate-like trabeculae which are oriented mostly parallel or perpendicular to the surface of the bone. The channels of

diploic blood vessels can be visualized. The inner table is thinner than the outer and imprints of blood vessels could be seen on its surface [26].

Micro-CT imaging of the frontal bone affected by HFI shows a slowly progressing chronic process with no alterations on the outer table and external part of the diploic region. No trabeculae could be differentiated in the internal part of the spongiosa. Endosteal proliferation or "bulges" are nodular in shape and could not be strictly demarcated. The overall density of the new tissue is increased. This was the first description of microarchitecture of HFI done by Rühli et al. [26] and it did not include any quantitative analysis (which we employed in our research). The authors outlined that a three-dimensional visualization could be very helpful in the evaluation of the size and type of HFI. However, they stressed that histological examination could be more useful regarding the texture of the bone which represents lamellar bone better and indicates that the reformation of the bone is not new [26].

In this research, we investigated and compared microarchitecture of the frontal bone between males with different types of HFI, as well as between males and females with HFI – in general aspect and with corresponding HFI types.

In a healthy bone, the trabeculae are arranged according to the main direction of strain. If no pattern is visible in the direction of the trabeculae, the pattern is called isotropic [67]. Our results have shown that in the spongiosa of males with different types of HFI there is an overall difference in the degree of anisotropy of trabeculae, which means that the trabecular pattern is different. However, this difference could not be proven among specific HFI subtypes. Therefore, this result does not imply any significant conclusion regarding microarchitectural discrepancies. This means that HFI subtypes in males could not be distinguished at the level of microarchitecture of the frontal bone. Similar results were shown in a study of microarchitecture of HFI in females [14]. We disagree with Rühli that micro-CT could be useful in the evaluation of HFI type [26], especially because their observation was made after scanning a single sample of one HFI type. It seems that the most widely used macroscopic classification of HFI, where types A–D are often regarded as "phases" or consecutive "stages" in the course of HFI development could not be supported at the microstructural level. On the other hand, we have shown that the thickness of the frontal and temporal bones were positively correlated with the severity of HFI in males. These contradictory results

could be explained in two ways: (1) the increase in thickness does not affect bone microarchitecture (e.g. all three layers of the frontal bone are affected equally by the process that follows the normal bone remodeling) or (2) morphological measurements of the skull were carried out on a much larger sample, allowing correlation to reach statistical difference.

Males with HFI tend to have an increased bone volume fraction of the total frontal bone sample compared to females with HFI, but this difference was not statistically significant. They did have an increased bone volume fraction in the outer table (as expected, followed by decreased porosity) which we attributed to sex dimorphism. There is a radiological study conducted on autopsy material with subjects without HFI showing that males have thicker diploic region than females; however, the total frontal bone thickness was not different [89]. On the other hand, in females with HFI bone volume fraction in the spongiosa is greater when compared to healthy females [14]. In accordance with all of the above, maybe our results could imply that the etiological factor contributing to HFI has a more prominent (or faster?) impact among females than among males, eventually leading to equal bone fraction in the diploic region. However, when comparing the corresponding HFI subtypes between sexes we did not find any relevant differences (except for the fractal dimension in the spongiosa of HFI type C, which does not have a true physiological significance for the flat bones).

Therefore, based on our results we have concluded that the microarchitectural structure of the frontal bone with HFI is the same in both males and females, in general aspect and with different HFI subtypes. Based on the data from anatomical examination, histological sections and CT scans, Hershkovitz stated that the same process underlay the bony metamorphosis in both sexes, underlining that the discrepancy in the frequency and magnitude of HFI manifestation might simply represent differential susceptibility to the causative factors [7].

Our results clearly demonstrate that in both sexes HFI is also accompanied with a slight thickening of other skull bones. Hence, HFI seems to affect other skull bones, and not exclusively the frontal bone, but in a different manner, making them slightly (but still statistically significantly) thicker. The thickening of other skull bones does not

have the morphology typical of HFI (e.g. bony island and protuberances) and, therefore, it should not be mistaken for HCD.

The hypothesis regarding the altered sex steroids as etiological factors in HFI formation relies on the effects of these hormones on bone metabolism. There are experimental studies where the effects of estrogen and androgen molecular signaling pathway were examined in relation to the cranial suture closure [90,91]. Estrogen receptor gene expression is associated with physiologic cranial suture fusion and intact signaling is necessary for normal mouse cranial suture fusion [90]. This study was carried out on mouse prefrontal suture, which is analogous to human metopic suture. The midline of the frontal bone (the region of metopic suture) is not affected by HFI and these results have to be interpreted with caution when extrapolated to humans, but they could support the fact that the frontal bone is a "favored endocrine target" as Hershkovitz suggested [7]. On the other hand, it has been shown that androgen stimulation of dural cells and osteoblasts isolated from human fetal calvaria promotes cell proliferation and osteoblastic differentiation and can induce cranial suture fusion [91].

However, the question remains whether the etiological factor leading to HFI affects *only the skull* or there are some measurable effects on other skeletal sites. Bone loss and consequent osteoporosis occurs with aging throughout the skeletal system, especially in females who are in menopause [43,45]. Those females are also most commonly affected by HFI [7]. It is now well-established that estrogen deficiency plays a role in the development of osteoporosis in males as well [44]. If, for example, increased exposure to estrogen was the cause of HFI, we would expect increased bone density or slower bone loss during aging to be present, apart from the thickening of the frontal bone (and apparently, other skull bones). To examine this, we decided to conduct densitometric measurements at one of the standard sites (proximal femora) in both sexes. This was the first step, followed by further analysis of the femoral microarchitecture.

Bone comprises specialized cells and mineralized, as well as nonmineralized connective tissue matrix (osteoid) and together they form cortical and cancellous (trabecular) structures. Cortical bone is relatively solid and compact and represents ~80% of the skeleton. Cancellous bone has a honeycomb-like appearance and consists of interconnected plates and strands. Bone is formed and removed by two highly specialized and terminally differentiated bone cell types: osteoblasts, which are responsible for the deposition of new bone matrix and its mineralization, and osteoclasts, which are uniquely capable of resorbing the mineralized matrix [92]. This is a highly dynamic tissue that responds and adapts to changes in systemic signals, including hormones [43].

Although there is correlative evidence of relationships between weak adrenal androgens [93] or progesterone [94] and bone mineral density (BMD), direct evidence from interventional studies in humans is inconsistent regarding these steroids. In the regulation of bone metabolism by steroid hormones, the focus remains on estrogen and testosterone.

The effects of estrogens and androgens on bone are exerted upon binding with high affinity to the estrogen receptor (ER) α and β and the androgen receptor. Osteoblasts and osteoclasts contain both ERs, although their concentration is lower than in reproductive tissues [95]. ERα is predominantly localized in the cortical bone, and ERβ in the trabecular bone [96]. Global ER-deletion animal models have shown that: (1) the loss of ERα compromised cortical bone thickness in both sexes and (2) ERβ did not regulate bone metabolism in males but could either compensate for loss of ERα in females, at least in the setting of elevated estrogen levels, or when ERα was present, ERβ appeared to antagonize ERα action on bone [97]. In both sexes, estrogen plays the central role in the regulation of bone metabolism by conserving bone mass, suppressing bone turnover and maintaining balanced rates of bone formation and resorption [92,98]. It suppress bone resorption in trabecular and endocortical bone surfaces by decreasing osteoclast numbers, through attenuation of their differentiation and shortening their life span by stimulating apoptosis [98]. The major effect of testosterone on bone metabolism is the reduction of bone resorption, in addition to increasing the lifespan of both osteoblasts and osteoclasts [92]. Testosterone acts mainly indirectly, via aromatization of testosterone to estrogen. Androgen suppression is potentiating the effects of estrogen

on bone metabolism [93,99]. The decrease in bioavailable E2 in males with advancing age is associated with a decrease of bone mass. Therefore, in both sexes estrogen deficiency contributes to profound and sex monomorphic effects of aging on skeletal involution and the development of osteoporosis in humans [45].

Environmental estrogens (xenoestrogens) represent a heterogeneous class of chemicals, both man-made and natural, with estrogen-mimicking activity [44]. Xenoestrogens will not necessarily reproduce estrogen effects, but rather initiate distinct and often nonpredictable responses that differ between end organs [100]. As a result, low doses of xenoestrogens can interfere with natural estrogen actions, even in the presence of higher circulating estrogen concentrations. Given the fact that they are widely used in modern society [101], we should not ignore their potential influence on bone health.

DXA is the most widely available and most commonly utilized method for BMD evaluation and still represents a gold standard for clinical diagnosis of osteoporosis [102]. BMD measurement is a surrogate for the measurement of bone strength [103]. Femoral neck has been considered the most reliable region because the measurement of femoral neck BMD is less influenced by degenerative changes and the position of the leg [104].

To test the hypothesis that HFI affects other skeletal sites apart from the skull, we first analyzed densitometric parameters of femora in males and females with HFI and compared them with subjects without HFI.

Males with HFI tend to have increased values of DXA parameters in femoral neck and total femoral sample, with borderline significance when compared to the age-matched males without this condition. Moreover, when we compared males without HFI, males with milder HFI (types A and B) and severe (types C and D) HFI, we noticed that the mean values of BMC and BMD in both observed regions tend to increase with HFI severity. After the adjustment for height and weight these small differences remained and were more pronounced between males with milder and severe HFI. It has previously been shown in a clinical study that serum estradiol was positively correlated with the femoral neck BMD of elderly Chinese males [105]. Another clinical study proved that the combination of low E2, low testosterone and high sex hormone-

binding globulin in males is associated with significantly faster rates of BMD loss [106]. Although men also develop osteoporosis with aging, they lack the abrupt cessation of gonadal function present in females and true “andropause” does not exist [44]. Total testosterone levels only slightly decline with age, and in the majority of elderly men testosterone levels are maintained above the threshold that separates normalcy from symptomatic hypogonadism [44]. Since our subjects were age-matched, we believe that these densitometric differences (even without reaching significance) might be the result of discrete hormonal imbalance rather than be age-induced.

On the other hand, females with HFI had slightly lower DXA parameters than their age-matched female controls, which again have not reached statistical significance. There was no correlation between any DXA parameter and HFI severity. After adjustment for height and weight, females with severe HFI had the lowest values of BMC and BMD in both observed regions. The results from a very similar study were also statistically insignificant, although the trends were different: females with the severe form of HFI had the highest values of BMD in neck, total hip and vertebral region, compared to those with moderate HFI (types A–C) and females without HFI [59]. The differences between our results could be caused by the discrepancy in mean age (their sample was 10 years younger), which is not an irrelevant factor when comparing bone quality in postmenopausal females.

Literature data suggest that BMD differences between sexes are much smaller than expected or even absent at certain sites, especially following adjustment for bone size [107,108]. Peak bone mass in males appears to be site-specific and mainly due to greater bone area. In other words, males develop greater peak bone mass because their bones are longer and wider, but not denser [45]. For these reasons, we decided to compare densitometric, HSA and femoral microarchitectural parameters between males and females with HFI and, being aware of biological differences, we took into account only the adjusted data.

It turns out that males with HFI have significantly higher BMC only in the neck area, but since they also had proportionately greater femoral neck area, as expected, there were no changes in BMD (in femoral neck or regarding the total sample). This means that males and females with HFI have similar femoral bone mineral density. It is important to note that these data are also adjusted for age (in a corrected model the

subjects' age was 68), so we can exclude age the difference between males and females as a confounding factor for BMD.

Hip structure analysis (HSA) incorporates the principles of mechanical engineering into an analysis of bone mineral data acquired using a conventional bone mineral scanner like DXA [66]. HSA allows the measurement of geometric contributions to bone strength in the proximal femur. In addition to DXA measurements, we also conducted HSA in males and females with and without HFI to obtain a better insight into femoral geometry and strength which could possibly be related to HFI as a systemic condition.

During lifetime long bones are loaded in both axial compression and in bending. In axial compression, the forces are distributed fairly uniformly over the mineralized bone surface. Bone's ability to resist axial compressive forces is directly proportional to the mineralized bone surface cross-sectional area (CSA). Therefore, CSA is highly correlated with BMC measured on DXA scans [65,103]. Our results demonstrated that even though males with HFI had better BMC values in the femoral neck region (although without reaching statistical significance), they had lower CSA values in the same region and greater CSA values in the intertrochanteric region compared to males without HFI. Adjusted data have shown that males without HFI have greater CSA values in the narrow neck region compared to males with milder and severe HFI, which proved to be statistically significant. Basically, this could mean that regardless of the fact that "healthy" males have slightly less bone mineral content and density, their femora are still more resistant to axial compression, at least at the femoral neck. In the intertrochanteric region, males with HFI appear to be more resistant to axial compressive forces. If we assume that HFI does have an impact on increased bone formation at other skeletal sites (reflected as higher femoral BMD), our results have shown that, for some reason, that did not imply overall greater bone strength. We expected that males with HFI would have stronger bones due to possible systemic effect of this condition. According to our DXA and HSA results, they might be more prone to femoral neck fracture, but protected against fracture in the intertrochanteric region. The etiological factor that leads to HFI maybe has varied influence on different femoral regions or this could be due some other unrelated reason. In this study, there were no

subjects with fractures, which is why further research is needed to confirm whether males with HFI are at greater risk of fractures than their age-matched healthy controls.

Statistical significance has also been found in CSA values among females. Females without HFI have the highest CSA values in the narrow neck region and also the lowest values in the shaft region, compared to females with milder and severe HFI. In that sense, females with HFI will show greater resistance to compressive forces in the shaft region, but they might be more prone to femoral neck fracture due to axial compression.

An interesting finding was that, after the adjustment of data for age, height and weight, there were no differences in CSA values between males and females with HFI. Axial compression of the skeleton primarily originates from body height and weight [103] and it was expected that CSA values would change. As with DXA measurements, this result proves that the resistance to axial compressive forces in persons with HFI remains the same regardless of sex.

In addition to being loaded in axial compression, long bones are also loaded in bending. Section modulus (SM) is a measure of bending and torsional strength of the bone [65,66,109]. SM is dependent of cortical thickness – small increases in the outer radius (periosteal thickness) have a much greater effect on the cross-sectional moment of inertia (which is a function of SM) than relatively larger increases in the inner radius (endocortical thickness) [103]. Periosteal bone apposition is responsible for the enlargement of bones during growth, where it is likely that testosterone plays an important role, directly or indirectly (via the growth hormone/insulin-like growth factor) [45]. Therefore, compared to females, males have a greater cortical bone diameter due to greater periosteal apposition, placing the cortex further away from the neutral axis [45]. There were no changes in SM when comparing the three male groups, although we have noticed some trends: males with severe HFI tend to have the highest SM values in the narrow neck region, and males with milder HFI in intertrochanteric and shaft region. Males with severe HFI also had the greatest (total) cortical thickness in the narrow neck and intertrochanteric region, while healthy males had the greatest thickness in the shaft region (none of this proved statistically significant). This could mean that, all together, males with HFI might have at least slightly greater rate of periosteal apposition in the femoral neck and intertrochanteric region. In one study, the addition of testosterone to

estrogen replacement in a male with mild hypogonadism resulted in incremental gains in cortical expansion and thickness compared with estrogens alone [110] and this supported the model in which optimal periosteal bone expansion during growth requires both androgen receptor and ERα actions [111]. As far as we know, none of our male subjects with HFI were on estrogen replacement therapy or in a period of extensive growth (only two of them were in the 21–30 age group) and if we assume that they had normal testosterone levels, this could maybe imply that greater than normal estrogen levels are partially responsible for their slightly greater periosteal apposition.

Among females, there was also no statistical significance in the values of SM or cortical thickness. As expected, males with HFI had better SM compared to females with HFI, but it was unexpected that after the adjustment of data these differences would remain significant only in the narrow neck region. Compared to females, males usually show increased SM values in every investigated femoral region, in all age groups, before and after adjustment for body size, due to a greater rate of periosteal apposition and smaller rate of endosteal resorption [59,109,112]. In the intertrochanteric and shaft region, males and females with HFI obviously show similar bone resistance to bending forces, at least in our study sample.

Cortical instability may occur when excessive cortical thinning is present even though the remaining bone mass has been redistributed toward the periphery of the cross-section. In HSA, this is reflected through the buckling ratio (BR), which is the ratio of periosteal thickness to total cortical thickness and it basically represents bone instability due to thinning of the cortical bone [65,103]. BR ratios above 10 are considered to be correlated with abrupt loss of bone strength [103]; however, this observation is made based on the engineering model of the hollow tube [66] and could only be partially extrapolated to long bones like femur. There were no statistically significant differences in BR between the three groups investigated in our study, in either male or female sample. However, BR was the highest in the narrow neck (>10) in all groups, which was most pronounced in subjects with severe HFI. BR significantly increases with aging [103,109,112]. Even though the severity of HFI did not correlate with age in our study, the majority of severe HFI was found in subjects above 50 and we believe that aging is the main reason for high BR among subjects with severe HFI. Males and females with HFI also had similar BR, which is in accordance with other

studies conducted on patients without HFI [113]. In conclusion, it appears that in both sexes the presence of HFI did not affect bone instability due to cortical thickening.

One of the privileges of this study was the ability to take bone samples from subjects (cadavers) which allowed us to access bone microarchitecture via micro-CT imaging, similarly to the use of quantitative computed tomography (QCT) in clinical setting. In the final part of our analysis, we wanted to compare femoral microarchitecture between males and females with and without HFI.

Probably the most important finding of this research was the detection of significant changes in femoral microarchitecture in subjects with HFI. In males, differences were noted only with the trabecular bone – males with HFI had denser trabecular bone (increased bone volume fraction) with trabeculae that were closer together (decreased trabecular separation) compared to males without HFI. They also had slightly thicker trabeculae with more isotropic orientation (smaller DA), but these results have not reached statistical significance. However, when we divided them into three groups (without HFI, milder and severe HFI), the statistical analysis showed that males with milder HFI had the most "plate-like" trabeculae (the lowest SMI) compared to other two groups. Using Spearman's correlation we showed that the more severe HFI was, the closer the trabeculae were.

Females with HFI had increased cortical bone volume fraction and, as expected, their cortical bone was less porous compared to females without HFI. These differences diminished and new ones emerged when we divided them into three groups. Females with milder HFI had the highest number of trabeculae which were also better connected to each other. Overall intergroup difference was also evident in relation to pore diameter, but this proved insignificant when comparing individual groups. The correlation between microarchitectural parameters of the cortical bone and the presence/severity of HFI almost reached significance for cortical bone volume fraction and porosity and, in our opinion, this was probably due to a relatively small sample size. It is important to note that aside from trabecular thickness in females, none of the investigated microarchitectural parameters correlated with age in either sex. That means that we can exclude ageing as the causal factor behind the observed changes.

Interesting results emerged when we compared microarchitectural parameters of the femoral bone between males and females with HFI. If we observe unadjusted data, it looks like the trabecular bone in males has an increased degree of anisotropy, which indicates that trabeculae are more aligned to one direction, corresponding to a principal stress direction [114]. Their trabeculae are also more "plate-like", but poorly connected to each other. We have previously explained that due to different body size and, in accordance with our research goals, we can compare males and females only after adjustment for age, height and weight. After adjustment, it is becoming apparent that there are no microstructural changes of femoral bone in subjects with HFI, regardless of sex, neither in the trabecular nor in the cortical compartment. That means that, at the level of femoral bone microarchitecture, males with HFI have more parameters in common with females with HFI than with their healthy controls. Microarchitectural structure of the frontal bone is also the same in males and females with HFI, both in general aspect and with corresponding HFI types. Altogether, our results do not only indicate that HFI is most probably a *systemic phenomenon*, but also a phenomenon that affects *both males and females* in a similar manner.

We have demonstrated that subjects with HFI, males and females, tend to have "more favorable" values of bone microarchitectural parameters compared to the ones without this condition, at least in the region of the lateral femoral neck. Of note, lateral neck is considered to have poorer microarchitectural bone quality profile than medial neck, especially in males [114]. It would be interesting to investigate the microarchitecture of the medial neck as well; maybe these differences would be even more pronounced.

It has previously been shown that females with HFI had greater than normal BMC and bone width of the radius [42]. We have also shown that there is good correlation between femoral BMC and severity of HFI in males. All these data imply that the same etiological factor probably leads to bone formation on the frontal bone, at the same time inducing changes in trabecular and cortical bone at other skeletal sites. If we consider the altered sex hormone ratio to be the most probable cause of HFI, some interesting literature data on compartment-specific effects of estrogens and androgens on bone in females and males could support our microarchitectural analysis results. There is a possibility that the loss of the ligand (estrogen) may lead to altered bone mass

through a different mechanism than loss of the receptor [115]. This observation made us consider that compartment-specific changes of femoral microarchitecture in subjects with HFI (more pronounced with the trabecular bone in males and with the cortical in females) could be caused by the alteration of circulating levels of sex hormones *or* the altered expression of sex hormone receptors.

As we mentioned before, both ERα and ERβ are expressed in the trabecular bone, whereas the cortical bone mainly contains ERα [116,117]. In male mice, global ERα loss decreased bone turnover and cortical bone volume and increased trabecular bone volume [97,118], which is similar to our findings in males. In contrast, loss of ERβ did not affect bone development or homeostasis [97]. This suggests that in the absence of one ER, the other might compensate. It is noteworthy that rodent bone phenotypes do not completely agree with human phenotypes [44]. However, Smith et al. described the skeletal phenotype of a 28-year-old male with homozygous mutations in the ERα gene [119], which means that humans can also have nonfunctional ER due to genetic mutations. This individual had normal testosterone and elevated estrogen levels, unfused epiphyses and osteopenia.

Literature data imply that the signals of estrogens and androgens are orchestrated and fine-tuned at different bone anatomical sites, where they can be modified and integrated at different environmental cues (mechanical strains or the local concentration of paracrine cytokines and growth factors) [45]. Numerous observational studies have documented that the serum estrogen levels in males were more closely correlated with BMD and bone turnover markers than the serum testosterone levels [43]. In contrast to estrogen, evidence that the low circulating testosterone levels present in females have a significant impact on bone metabolism is weak or inconsistent [120]. In their extensive review regarding the regulation of bone metabolism by sex steroids, Khosla et al. proposed a working hypothesis for the compartment-specific effects of estrogens versus androgens on bone in females and males [43]. In healthy females, estrogen levels are sufficiently high so that estrogen consistently suppresses bone remodeling in both the cortical and trabecular bone, with testosterone playing a minimal role in the trabecular bone and no discernable role in the cortical bone. In that sense and in consistence with our results, females with HFI probably do not have abnormal androgen metabolism, but rather, as it was suspected all along, some sort of estrogen

surplus during lifetime. Healthy males, however, have much lower estrogen levels which are able to suppress cortical bone remodeling and perhaps have some effects on the trabecular bone, but they require substantial androgen action in the trabecular bone to adequately restrain bone remodeling and prevent bone loss in that compartment [43]. Having this in mind and in accordance with our results, it is possible that males with HFI show at least a slight increase in estrogen levels. However, their testosterone levels might be normal or even increased. This would not be supported by case reports regarding HFI in males (Table 1); however, case reports are still near the bottom of the evidence-based medicine pyramid. Indeed, we have not yet encountered males with HFI that had undisputed autopsy findings consistent with hypogonadism (except for a few cases of gynecomastia). Since bone structure alterations in subjects with HFI are clearly evident only at the microstructural and not at the macroscopic level, it could also be possible that the hormonal level changes are either quite discrete or their effect just does not last long enough because they start relatively late in life. We can also speculate that xenoestrogens could have some influence in each of these scenarios.

Establishing the identity of the deceased person is one of numerous tasks of forensic pathologists. If all soft tissues are absent, identity is established upon anthropological examination of the skeletal remains and the recognition of any pathological or anatomical abnormalities in bone. In forensic pathology, the procedure for identifying human skeletal remains falls into two distinct sections: (1) assigning the bones to general categories based on absolute criteria concerning whether they originate from humans or not, as well as sex, stature and age of those human remains and (2) comparative studies, where the remains are matched against antemortem data derived from those people who might be potential victims [121]. Identification of the human skeletal remains is also en expertise of anthropologists.

In forensic anthropology, morphological and metric methods which compare the shape and status of bones and teeth are considered advantageous because they are faster and less costly than, for example, DNA identification [122]. The determination of sex is the most important criterion, as it immediately excludes approximately half the population, whereas age, stature, and ancestry each provide points within a wide range of variables [121]. In general, robusticity tends to characterize male and gracility female

skeletons [123]. The postcranial skeleton (pelvis in particular) is considered a better indicator for sex assessment than the skull [60,61]. However, some agree that the perception of the skull as the second best estimator of sex persists despite evidence to the contrary [124,125]. It is also important to note that not all forensic cases provide the luxury of a complete skeleton and, more often than not, only skulls and several long bones are found.

The female skull is smaller, rounder and smoother than the rugged male. Muscle ridges are more marked in male skulls, especially in occipital areas where larger muscles are attached to the nuchal crests and in temporal and mandibular areas for larger masseter and temporalis muscles. Forehead is high and steep in the female skull and has a more rounded infantile contour than the male skull. Supraorbital ridges and mastoid process are more marked in male skulls, whereas frontal and parietal eminences are more prominent in female skulls. Glabella is more marked in males and orbital margins are rounder and less sharp. Palate is larger and of a more regular U-shape in males, while the smaller female palate tends to be parabolic. The male skull has a large mandible with a squarer symphysis region; female jaws are more rounded and project less at the anterior point. The age and ancestry have a profound effect on all of the described features. These sex variations represent the “typical” Caucasian aged between 20 and 55 [121,122].

There have been studies which considered the possibility of using HFI as a method for estimating sex and age of skeletons [48,126]. This is not surprising, since HFI manifests significantly higher prevalence and severity in females and is also age-dependent, which we confirmed in this research. Hershkovitz and al. have also shown that HFI does not depend on geographical origin, since European Americans and African Americans exhibit similar rates of HFI [7].

When frontal bone with severe form of HFI (type C or D) is found, our results indicate that most probably it belongs to a female, older than 70 years of age. Similar to our results, May et al. stated that there was more than 32% chance that an unknown skull with major HFI was a female over 70 years old, while there is an 86.9% probability that a skull aged 70+ years with major HFI is a female [48]. Many researchers wanted to determine whether cranial vault thickness could be used as an indicator of sex and age, but no clear trends have emerged, and the results have been

somewhat conflicting [89]. We demonstrated that measuring the thickness of the skull bones in subjects with HFI would not be useful in predicting decedent's age and measuring the thickness of the frontal bone would not help in the estimation of sex. However, according to our results, if the skull has the temporal bone thicker than 6.5 mm or occipital bone thicker than 7.5 mm, it more probably belongs to a male decedent. Calculating cut-off values for the use of temporal and occipital bone thickness as sex indicators in subjects with HFI proved to be statistically significant in our analysis; however, the correlation coefficient and sensitivity/specificity ratio were not satisfying enough. Therefore, we recommend using these values with caution.

An autopsy study has the obvious limitation of its cross-sectional design, meaning that we examined bone samples from various subjects at various ages at death and could not follow the process of HFI pathogenesis in the same individuals. Only a longitudinal study (in living subjects) would allow a follow-up to determine if this is a truly progressive process. In that case, accessing bone microarchitecture would require bone biopsy or the use of QCT, which is both invasive and expensive.

In our methodology, we choose to use the classification proposed by Hershkovitz et al. [7], since this classification is most commonly utilized, allowing comparison with other studies. Any kind of visual classification leaves room for a certain level of subjectivity, which we tried to overcome by diagnosing and classifying HFI with two experienced forensic pathologists familiar with this phenomenon.

The results of this research support the theory that hormonal imbalance is the most probable cause of HFI. The data about hormonal status of our subjects were not available and in general post mortem measurements of hormones are not reliable for many reasons [121,122]. Only longitudinal, clinical, controlled trials with hormonal monitoring and consecutive head CT scans of patients with HFI could definitely confirm this theory. The impact of other clinical variables should also be tested.

HFI is a condition that affects both sexes. Males with HFI are younger and most commonly have milder forms of this condition (types A and B) with occurrence and severity that are not age-related. On the other hand, age is a predictor for HFI occurrence in females who have almost four times greater chances of developing the most severe HFI form (type D). The menopause could be the turning point for HFI

pathogenesis in females. In males, there are some other (still unrecognized) factors which act in synergism with hormonal disturbances leading to HFI occurrence.

HFI is most probably a systemic phenomenon that affects both males and females in a similar manner. Microarchitectural structure of the frontal bone is the same in both sexes, in general aspect and with corresponding HFI types. HFI type could not be distinguished at the microstructural level of the frontal bone. The bone formation is most pronounced on the frontal bone, but other skull bones are affected as well. Their thickening does not resemble morphology typical of HFI and does not affect cranial vault size in males. In females, this leads to a slightly smaller longitudinal diameter of the skull which most probably does not have clinical significance. The factor(s) causing HFI therefore affects different skull bones, not only the frontal bone.

It seems that apart from the skull, the same etiological factor behind HFI induces changes at the level of bone microarchitecture at a remote skeletal site (femoral bone), in both sexes, regarding both quantitative parameters and spatial microarchitectural organization of the trabecular bone. However, these alterations still do not have the magnitude to induce obvious, straightforward overall increase of bone strength measured by conventional methods (DXA). According to HSA, both males and females with HFI could be more prone to femoral neck fracture due to axial compressive forces. Males with HFI could be protected against fracture in the intertrochanteric region and females in the shaft region. The etiological factor that leads to HFI may have varied influence on different femoral regions or this could be due some other unrelated reason.

At the level of femoral microarchitecture, males with HFI have denser trabecular bone with trabeculae that are closer together, while females have denser cortical bone which is less porous. However, there are no microstructural changes of femoral bone in subjects with HFI, either in the trabecular or in the cortical compartment, regardless of sex. Once again, this strongly suggests that HFI is most probably a *systemic phenomenon, with similar bone effects in both sexes.*

HFI could be used in forensic pathology as a supplementary to other established methods for estimating sex and age of unidentified human skeletal remains. This is especially useful if an anthropologist or forensic pathologist has only the frontal bone at disposal – the mere presence of HFI could be an additional criterion for positive identification.

6 Conclusions

1. HFI occurrence in males in not age-dependent. Males have the same risk of HFI occurrence as females below 55 years of age, but after this age HFI manifestation starts to be age-related only in females. The menopause could be the key event in the HFI pathogenesis in females.

2. Males with HFI tend to have milder forms (most common types are A and B) compared to females. The most severe form (type D) is rare in males. The severity of HFI does not correlate with age either in males or females.

3. Both males and females with HFI have thicker frontal, temporal and occipital bones. This implies that the factor causing HFI does not strictly act locally on frontal bones, but probably on other skull bones as well. In males, HFI does not affect cranial vault size and in females the longitudinal diameter of the skull is slightly decreased. However, clinical significance remains unclear.

4. In males, HFI type could not be distinguished at the level of microarchitecture of the frontal bone.

5. Microarchitectural structure of the frontal bone is the same in males and females with HFI, both in general aspect and with corresponding HFI types.

6. In males, bone mineral content and density in both observed regions (neck and total femoral sample) tend to increase with the occurrence of a more severe form of HFI. Bone mineral content and density in neck and total femoral sample didn’t differ among females without HFI and those with moderate and severe HFI types. Males and females with HFI could be more prone to femoral neck fracture due to axial compressive forces, compared to their age-matched controls. Subjects with HFI have microarchitectural changes of the proximal part of femora – males have denser trabecular bone with trabeculae that are closer together, while females have denser cortical bone which is less porous. This implies that HFI is probably a systemic condition.

7. After adjustment for age, height and weight, males with HFI had slightly increased bone mineral density in neck and total femoral sample compared to females with HFI, but without statistical significance. Compared to females, males with HFI show better resistance to torsion forces in the femoral neck; otherwise, there were no differences between subjects with HFI regarding hip structure parameters. There are no femoral microarchitectural changes in subjects with HFI, regardless of sex, either in the trabecular or the cortical compartment. HFI probably has similar systemic effects in both sexes.

8. Our results indicate that, when a skeleton with general markers of old age (e.g. osteoporosis, articular surface degeneration, osteophytes) along with the skull with severe HFI is found, it probably belongs to a female older than 70 years of age.

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Author's short biography

Dr. Danica Cvetković was born on March 30th, 1988, in Smederevo, where she finished elementary school and Gymnasium. In 2007, she enrolled at the School of Medicine, University of Belgrade, where she graduated in 2013 with the grade point average of 9.78/10. She started working as a Teaching Associate at the Institute for Forensic Medicine, School of Medicine, University of Belgrade, in 2015. Her residency in forensic medicine has begun in April, 2016. In the 2015/2016 school year, Miss Cvetković has started her doctoral studies within the Skeletal Biology module, carried out in English. In 2016, she completed specialist academic studies in Epidemiology at the same faculty. She is one of the associates on the project of the Ministry of Education, Science and Technological Development of the Republic of Serbia entitled "Functional, Functionalized and Enhanced Nanomaterials" (grant No. 45005) and member of the research team in the Laboratory for Anthropology of the School of Medicine, supervised by Professor Marija Đurić. Since 2017 Miss Cvetković has been working as a Teaching Assistant at the Institute for Forensic Medicine. She authored and co-authored 26 scientific publications, out of which 20 have been published *in extenso* in journals from the Journal Citation Reports (JCR) list.

Kratka biografija autora

Dr Danica Cvetković je rođena 30. marta 1988. godine u Smederevu, gde je završila osnovnu školu i prirodno-matematički smer Gimnazije. Medicinski fakultet Univerziteta u Beogradu upisala je 2007. i diplomirala 2013. godine sa prosečnom ocenom 9,78. Na Institutu za sudsku medicinu Medicinskog fakulteta u Beogradu zaposlila se kao saradnik u nastavi 2015. godine. Specijalistički staž iz sudske medicine započela je na Medicinskom fakultetu u Beogradu u aprilu 2016. godine. Doktorske studije na engleskom jeziiku, modul Biologija skeleta, upisala je na Medicinskom fakultetu u Beogradu školske 2015/2016 godine. Završila je specijalističke akademske studije iz oblasti epidemiologije na istom fakultetu 2016. godine. Saradnik je na projektu Ministarstva prosvete i nauke Republike Srbije „Funkcionalni, funkcionalizovani i unapređeni nanomaterijali“ br. 45005 i deo istraživačkog tima u Laboratoriji za antropologiju Medicinskog fakulteta, kojim rukovodi prof. dr Marija Đurić. U zvanje asistenta za užu naučnu oblast Sudska medicina izabrana je 2017. godine. Autor je ili koautor u 26 stručnih publikacija, među kojima je 20 radova štampano u celini u časopisima sa JCR liste.

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