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## Radiomorphometric indices of mandibular bones in an 18th century population



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### ABSTRACT

**Objective:** To estimate four radiomorphometric indices of mandibular bones in an 18th century population sample, and possibly associate the findings with bone mass loss related to sex, age, nutritional habits and pathologies reflecting on the bone.

**Design:** Thirty-six skulls (31 males, 5 females), recovered from the crypt of Požega Cathedral in Croatia were analyzed. Age estimation was based on tooth wear, and Eichner class was determined according to the number of occlusal supporting zones. The parameters in recording analogue orthopantomographs were set to constant current of 16 mA, exposure time of 14.1 s, and voltage between 62–78 kV. Films were processed in an automatic dark chamber processor for 12 min, and digitized at 8-bit, 300 dpi. The thickness of the mandibular cortex was assessed below the mental foramen (MI), at antegonion (AI), at gonion (GI). Qualitative mandibular cortical index (MCI) was assessed.

**Results:** Average values of MI, AI and GI were  $3.97 \pm 0.94$  mm,  $2.98 \pm 0.56$  mm, and  $1.99 \pm 0.55$  mm, respectively. Statistically significant differences between males and females were found for AI right ( $p = 0.014$ ), GI left ( $p = 0.010$ ) and GI average ( $p = 0.006$ ), and were in all cases higher in males. There were no statistically significant differences between age groups for either index ( $p > 0.05$ ). Considering Eichner classification the differences were not significant for MI ( $p = 0.422$ ), AI ( $p = 0.516$ ), and GI ( $p = 0.443$ ), but in Eichner classes II, MCI was significantly higher ( $p = 0.02$ ).

**Conclusion:** The obtained data does not suggest generalized malnutrition or calcium, phosphorus and vitamin D deprivation in the historic population studied.

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## 1. Introduction

The assessment of bone density in ancient skeletal remains is a very valuable method since it may indicate nutritional habits, vitamin deprivation and the presence of various pathologies reflecting on the bone pattern. According to the available literature densitometric studies on skeletal remains of various historic populations have been done, but not on jaws.<sup>1–3</sup> During the first three decades of life bone synthesis predominates, so bone mass is at its highest point in the second half of the third decade, and then bone mass decreases at a rate of less than 1%/year, except in women during menopause when it decreases at a higher rate.<sup>4</sup> On the individual level, the factors influencing bone mass include genetics, delayed menarche and hormonal imbalances (for example diabetes mellitus, hyperthyroidism, hypogonadism, hyperparathyroidism, Cushing syndrome can all lead to reduced bone mass), and physical activity.<sup>5,6</sup> The activity of muscles attaching to bones affects bone density. The greater the contractile capacity of a muscle and the greater the force it liberates on contraction leads to higher bone mass at sites of muscle attachment to the bone.<sup>6</sup> At the level of a whole population, either protein or protein-calorie malnutrition could be the underlying causes of decreased bone mass i.e. osteopenia.<sup>1,2</sup> Besides caloric intake, several vitamins A, D, C, and K, and micronutrients like calcium, magnesium, and zinc may affect bone formation.<sup>7</sup>

Radiographic assessment of jaw bones is of great value in estimating quality and quantity of other bones in the body, and based on particular radiographic finding, some systemic or local conditions could be presumed, such as resorption or osteoporosis. A number of quantitative and qualitative radiographic assessments of mandibular bones were introduced and used for this purpose, including densitometric and morphometric ones.<sup>8–10</sup> Widely used qualitative index of mandibular bone assessment is mandibular cortical index (MCI).<sup>11</sup> Mandibular cortical thickness measured at different points of the lower mandibular border represent quantitative indices.<sup>10,12–14</sup> Although using radiomorphometric indices in diagnosing low bone mass density (BMD) at the axial skeleton has been questioned, it is generally accepted as auxiliary method in identifying bone mass loss,<sup>15,16</sup> and in a number of studies orthopantomographs were used in recognizing patients with lower BMD as well.<sup>11,17,18</sup> Good correlation between mandibular and skeletal BMD has been reported: BMD measured by dual-energy X-ray absorptiometry (DXA) in the mandible was shown to be positively correlated with BMD in lumbar spine, femoral neck and forearm.<sup>19–22</sup> Also, it was reported that panoramic radiomorphometric indices are significantly correlated with mandibular BMD.<sup>19,20,23</sup>

The aim of this study was to assess mandibular cortical index (MCI), and perform the measurements of radiomorphometric indices (mental (MI), gonial (GI) and antegonial index (AI)) on a historic sample from the 18th century. Furthermore the aim was to compare the recordings according to sex, age, and the number of occlusal supporting zones.

## 2. Materials and methods

### 2.1. Sample selection

Osseal remains included 175 skulls. They were recovered in 2005 from the crypt of Požega Cathedral (Croatia) after the floor deteriorated. In the part of the crypt under the sanctuary priests were buried, and in the rest of the crypt members of respectable families and professions (judge, senator, physician, notary, organ player, craftsman, miller). In 1769 burial fee was 16 forints. It is interesting that the crypt was used as burial site long after the decree of Joseph II from 1784, by which he ordered closing of all the church crypts. In fact, the last burial was in 1867. During the 19th century room for coffins was made by removing the remains of old coffins and placing the human remains in three biers. When the crypt was entered in 2004–2005, we recovered the osseal remains belonging to the 18th century population from the biers.

The material was carefully cleaned and examined at the School of Dental Medicine at Zagreb University, and afterwards returned to the original site. Of the 175 skulls recovered, only 36 were chosen for densitometric analysis. The chosen specimens were older than 15 years i.e. had only permanent dentition, sex and age could be determined and vertical and horizontal dimensions were reproducible.

The age estimation was based on tooth wear analysis.<sup>24</sup> Sex estimation was made from the skulls alone, and was based on the shape of supraorbital ridges, nuchal crests, mastoid processes and muscular ridges. The specimens were further classified according to Eichner- dependent on the number of occlusal supporting zones.<sup>25</sup> Eichner recognizes four occlusal supporting zones: two in molar and two in premolar regions. There must be at least one intermaxillary contact in the zone for it to be counted. In class I (or A) there are contacts in all four supporting zones, in class two (or B) in less than four zones, and in class III (or C) there are no occlusal contacts. Class II is further subdivided into II-1 with three supporting zones, II-2 with two supporting zones, II-3 with one, and II-4 with anterior tooth contact but no supporting zones contact. There are three subclasses in groups I and III depending on the teeth missing.<sup>25</sup> Only Eichner's classes I and II were used in the investigation because horizontal and vertical dimensions could easily be reproduced. This could explain way the oldest group analyzed counted least individuals. Besides, the Lovejoy ageing method of assessing occlusal tooth wear tends to underage the older individuals.<sup>24</sup>

### 2.2. Radiographic examination

Before recording each orthopantomograph, mandible was fixed to the skull with self-adhesive tape. The skull was attached to a wooden stand at a height of 1.5 m, and position was the same for all specimens. The recording parameters were set to constant current of 16 mA and an exposure time of 14.1 s; the kV varied between 62 and 78 kV (Sirona model no. 5968573 D3 200; Siemens, Munich, Germany). Images were recorded using radiographic film (ORTHO CP-G PLUS Agfa; Agfa-Gevaert Group, Mortsel, Belgium). The films were processed in an automatic dark chamber processor (XR 24 Nova; Dürr Dental GmbH u. Co

KG, Bietigheim-Bissingen, Germany) for 12 min. The radiographs were then put on negatoscope, and digital photographs were made using Nikon D7000 (Nikon Corporation, Tokyo, Japan) at 8-bit, 300 dpi. Digital images were then analyzed and index values calculated by applying linear measurements on the images using Scion image (Beta 4.0.2.) software tools.

### 2.3. Radiomorphometric assessment

Radiomorphometric indices were assessed on all radiographs by one experienced observer (DKZ) and the measurement was repeated after one-month interval. There was no significant difference between the two measurements ( $p < 0.05$ , paired t-test).

The cortical widths on the lower border of the mandible were measured along three lines, as previously described.<sup>9</sup>

Mental index (MI) was measured along the line perpendicular to the mandibular lower border tangent, passing through the centre of mental foramen.

Similarly, gonion (GI) and antegonion (AI) indices were obtained by measuring cortical widths at lines perpendicular to the lower border tangent, passing through gonion (GI) or the point of intersection of the lower border tangent and the line best fitting the anterior border of the ascending ramus (AI) (Fig. 1).

Mandibular cortical index (MCI) was determined based on the appearance of the inferior cortex on radiographs, according to the criteria set by Klemetti et al.<sup>11</sup>: C1 – the endosteal margin of the cortex is even and sharp on both sides of the mandible; C2 – the endosteal margin has semilunar defects with cortical residues 1–3 layers thick on one or both sides; C3 – the porous endosteal margin consists of thick cortical residues.

### 2.4. Statistical analysis

The data were analyzed using SPSS ver. 17 (IBM, Chicago, IL, USA). T-test and chi-square were used in analyzing data where statistical significance level was 0.05. Due to an asymmetrical ratio of males and females, and a relatively small sample, bootstrapping method of difference of two medians was performed using Resampling Procedure software version 1.3

(David C. Howell, University of Vermont). Number of repetitions was 5000 and the level of significance 0.05.<sup>26</sup> (Table 1)

## 3. Results

Recorded radiomorphometric indices of mandibular bones in the population from the 18th century are shown in Table 2.

The sample counted osseal remains of 31 male and 5 female individuals. MCI, MI, GI and AI were recorded for both sides and average values of the indices were calculated. Correlation between the right and left sides was high for all variables with no significant differences between them ( $p > 0.05$ ).

Twenty one samples exhibited MCI class I at both sides, in seven samples MCI classes II were recorded on both sides, and in seven class I on one side and class II on the other side. MCI class III was recorded in only two samples (one female +45 years and one male 35–44 years).

Distribution of samples according to Eichner classification was as follows: 25 samples belonged to group I and 11 to group II. As for Eichner subclasses, 18 samples belonged to I-1: all teeth in occlusal supporting zones were present; 6 belonged to II-2: some teeth in one jaw were missing but intermaxillary contacts in all supporting zones were present; 1 belonged to I-3: some teeth in both jaws were missing, but contacts existed in all occlusal supporting zones, five samples belonged to group II-1, and six to group II-2.

The average values of MI, AI and GI were  $3.97 \pm 0.94$  mm,  $2.98 \pm 0.56$  mm, and  $1.99 \pm 0.55$  mm, respectively. Statistically significant differences between males and females were found for AI right ( $t = 2.601$ ,  $df = 34$ ,  $p = 0.014$ ), GI left ( $t = 2.714$ ,  $df = 34$ ,  $p = 0.010$ ) and GI average ( $t = 2.963$ ,  $df = 34$ ,  $p = 0.006$ ), and were in all cases higher in males (Fig. 2).

There were no statistically significant differences between age groups for either index ( $p > 0.05$ ) (Fig. 3).

Considering MCI, the differences were not significant between males and females ( $\chi^2 = 2.54$ ,  $df = 2$ ,  $p = 0.281$ ) and age groups ( $\chi^2 = 4.306$ ,  $df = 6$ ,  $p = 0.635$ ).

Considering Eichner classification the differences were not significant for MI ( $\chi^2 = 36$ ,  $df = 35$ ,  $p = 0.422$ ), AI ( $\chi^2 = 31.02$ ,  $df = 32$ ,  $p = 0.516$ ), and GI ( $\chi^2 = 33.5$ ,  $df = 33$ ,  $p = 0.443$ ), but in

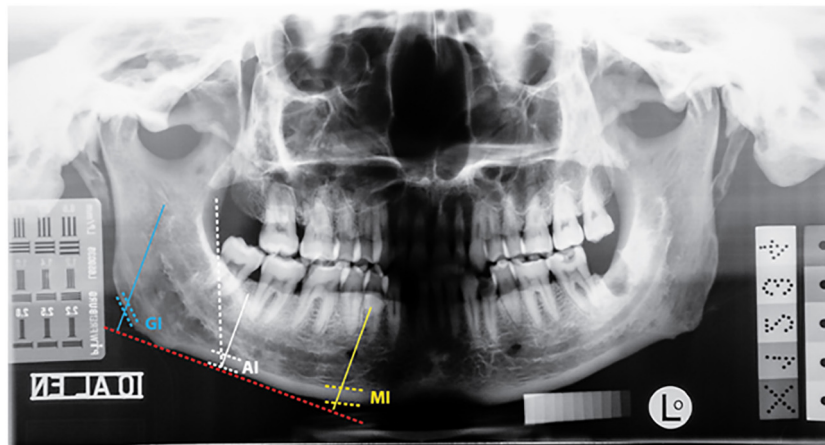


Fig. 1 – The assessment of MI, AI and GI along lines perpendicular to the inferior mandibular border tangent.

**Table 1 – Age and sex distribution of the sample.**

Age	<25	25–34	35–44	45+	Total
M	13	8	6	4	31
F	0	3	2	0	5
Total	13	11	8	4	36

Eichner classes II, MCI was significantly higher ( $\chi^2 = 7.845$ ,  $df = 2$ ,  $p = 0.02$ ).

Further analysis using bootstrapping technique, performed due to an asymmetrical ratio between males and females, revealed no differences between them in all variables ( $p < 0.05$ ).

#### 4. Discussion

It is well established that bone mass decreases with age, especially in women leading to the decrease of bone mass (osteopenia) with the increase of bone fragility (osteoporosis).<sup>4</sup> There are also other factors that influence bone mass, such as nutritional status and muscle activity, which make bone mass assessment especially interesting in the studies of historical populations, because conclusions about lifestyle can be made. According to the available literature there are no studies dealing with the assessment of either bone mineral density or radiomorphometric indices on jaws of historic populations.

Bone histomorphometry is considered a golden standard in determining bone mass in anthropological studies. It is, however, a destructive method, and the attempts were made to introduce noninvasive methods. Double-energy X-ray absorptiometry (DEXA) is the most widely employed method in determining BMD and diagnosing osteopenia and osteoporosis

in clinical settings because it is noninvasive and a high correlation between BMD and actual bone mass has been reported.<sup>27</sup> However, it is not as reliable on historic samples<sup>1,28</sup> where the BMD assessed by DEXA and bone mass assessed histomorphometrically were poorly correlated. Kneissel et al.<sup>28</sup> suggest that this can be attributed to the deposition of mineral salts into bones leading to a denser mineral phase, which could strongly influence X-ray-based density measurements, along with the lack of soft tissue and the distorting effect of the air entrapped within the cancellous bone.<sup>29</sup>

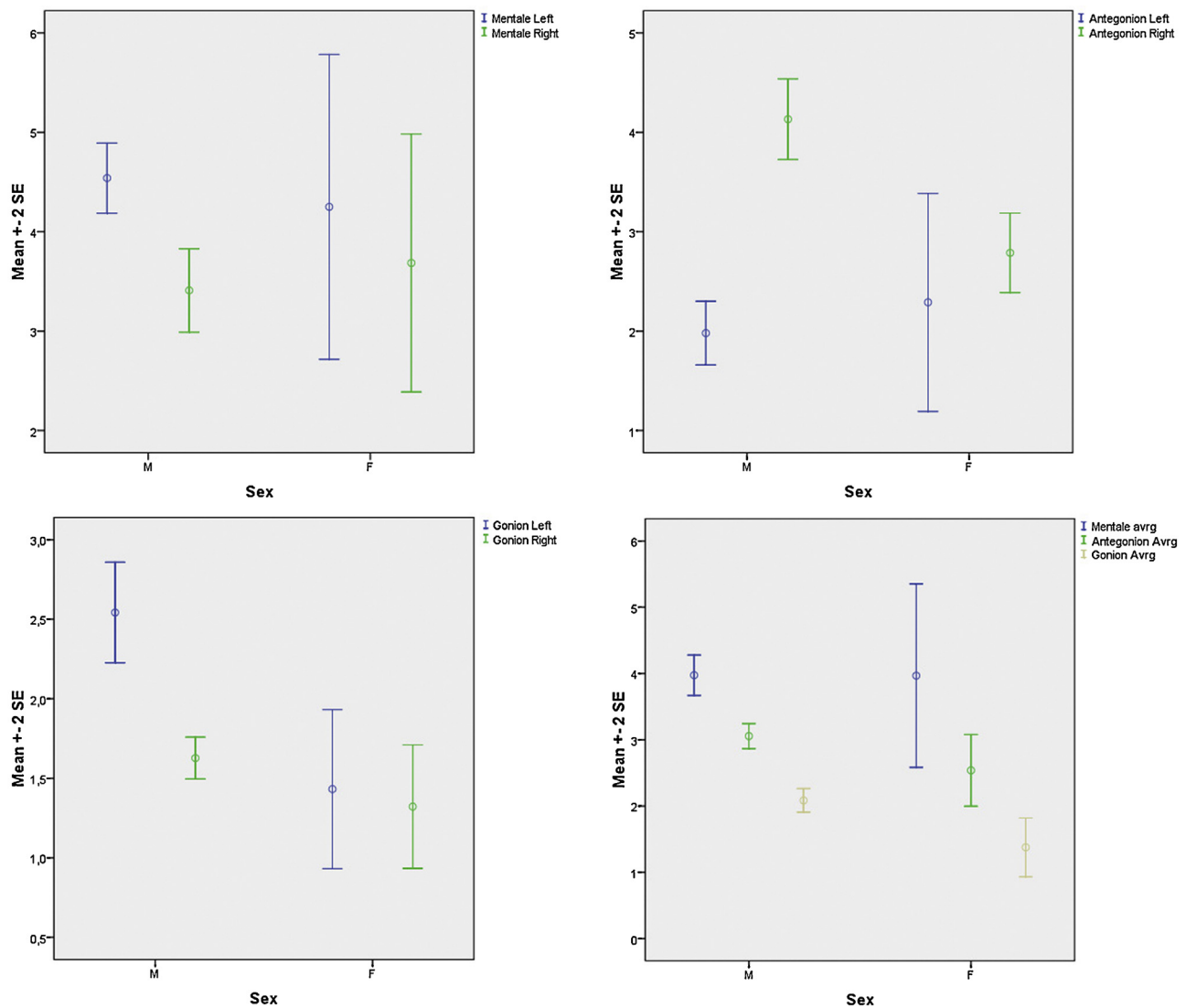
We therefore chose to use radiomorphometric measures on mandibular bone as more relevant method in estimating bone mass in an 18th century population, since correlation between radiomorphometric indices and BMD has been reported for the present populations,<sup>23</sup> and it is accepted that the shape and thickness of mandibular cortex on orthopantomographs, expressed by mandibular radiomorphometric indices, could be used as tools in identifying the higher possibility of bone mass loss further confirmed by densitometry.<sup>30</sup> Although the repeatability of panoramic radiomorphometric indices was questioned,<sup>23</sup> their efficacy in detecting bone mass loss was reported satisfactory.<sup>11,13,14,31</sup>

Bone loss starts at about the age of 35 and continues at different rates throughout life and depends on sex, site and age.<sup>18,19</sup> We did not find any statistically significant differences between age groups for either index. This finding was not in correlation with the results obtained by Devlin H et al.<sup>18</sup> who reported that the bone mineral densities of mandibular body was significantly related to age, also Drage NA<sup>32</sup> reported that bone mineral density of ramus was significantly related to age. Nevertheless, Gulsahi et al.<sup>15</sup> also did not find differences in mandible according to age. This might be explained by relatively small number of individuals in older groups, and the specificity of the dentition based ageing method that tends

**Table 2 – Values of radiomorphometric indices in males (M) and females (F) for mentale (MI), antegonion (AI) and gonion index (GI), on both sides of the mandible.**

	Sex	N	Mean	Std. deviation	Std. error mean
Mentale left	M	31	4.54	0.982	0.176
	F	5	4.25	1.714	0.767
Mentale right	M	31	3.4097	1.16763	0.20971
	F	5	3.6860	1.45039	0.64863
Mentale avrg.	M	31	3.9748	0.84850	0.15239
	F	5	3.9680	1.54828	0.69241
Antegonion left	M	31	1.98	0.892	0.160
	F	5	2.29	1.226	0.548
Antegonion right	M	31	<b>4.132</b>	1.1299	0.2029
	F	5	<b>2.788</b>	0.4467	0.1998
Antegonion avrg.	M	31	3.056	0.5248	0.0943
	F	5	2.539	0.6037	0.2700
Gonion left	M	31	<b>2.5426</b>	0.88059	0.15816
	F	5	<b>1.4320</b>	0.55971	0.25031
Gonion right	M	31	1.6271	0.36593	0.06572
	F	5	1.3220	0.43425	0.19420
Gonion avrg.	M	31	<b>2.0848</b>	0.49592	0.08907
	F	5	<b>1.3770</b>	0.49417	0.22100

The bold values of AI right, GI left and GI average were significantly different between males and females.



**Fig. 2 – Statistically significant differences between males and females were found for AI right, GI left and GI average. The indices were higher in males in all cases.**

to underage older individuals.<sup>24</sup> In the samples included in our study, vertical and horizontal relation could be reproduced with the purpose of minimizing differences in position during recording. Thus, besides excluding individuals with reduced occlusal support, which are logically older, the employed ageing method could additionally explain why the oldest groups we analyzed counted fewer individuals than the younger two groups.

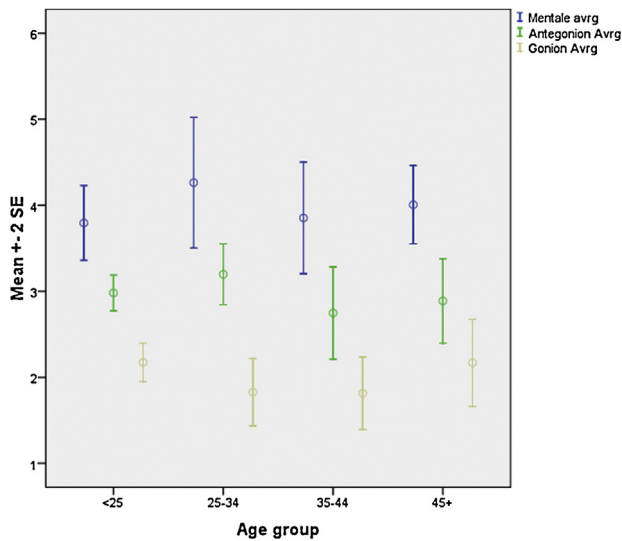
The fact that there were only five females and 31 males in our sample could be explained by the specificities of the whole sample from the Požega cathedral crypt. Namely, the whole sample consisted of 104 adults of whom 39 were females and 65 males. Furthermore antemortem tooth loss was significantly higher in females: it was 30.55% in females and 13.70% in males.<sup>33</sup> Since we chose samples where vertical and horizontal relation could be reproduced for our radiomorphometric study, only classes I and II according to Eichner, i.e. individuals with more teeth were selected, and we ended with only 5

females. Also, richer classes and priests were buried in the crypt. This could explain why males predominated.

The differences were significant between males and females considering AI right ( $t = 2.601$ ,  $df = 34$ ,  $p = 0.014$ ), GI left ( $t = 2.714$ ,  $df = 34$ ,  $p = 0.010$ ) and GI average ( $t = 2.963$ ,  $df = 34$ ,  $p = 0.006$ ), and were in all cases higher in males. This might be explained with the hormonal differences between males and females resulting in more pronounced and faster bone mass loss in females. Nevertheless, our sample counted only five females, so we could not make firm conclusions. In fact, additional resampling revealed no differences between males and females considering all radiomorphometric indices ( $p > 0.05$ ).

In our study the differences were not significant between males and females ( $\chi^2 = 2.54$ ,  $df = 2$ ,  $p = 0.281$ ) and age groups ( $\chi^2 = 4.306$ ,  $df = 6$ ,  $p = 0.635$ ) considering MCI. Ledgerton et al.<sup>13</sup> reported that the MCI has an excellent reliability and repeatability. By others the sensitivity and specificity of MCI





**Fig. 3 – There were no statistically significant difference between age groups for MI, AI and GI.**

in detecting bone mass loss was reported to be relatively low when compared to other indices,<sup>18,30</sup> yet we expected to find some differences between males and females and age groups considering MCI. Knezović Zlatarić et al.<sup>9</sup> reported that significantly more individuals with C3 cortex belonged to the older age group, in which there were significantly more females. The absence of statistically significant differences between sex and age groups in our study might again be attributed to the small number of females. It can also be explained by a sample of relatively young individuals where the differences related to postmenopausal hormonal changes in females could not have affected the bones' condition. Anyway, it was interesting to find that MCI was significantly higher where the number of occlusal supporting areas in premolar and molar regions was lower ( $\chi^2 = 7.845$ ,  $df = 2$ ,  $p = 0.02$ ), i.e. the samples belonging to Eichner class II were more likely to have semilunar defects on the inferior mandibular cortex or cortical residues on endosteal margin. This finding is consistent with the reported significant correlation of MCI and other radiomorphometric indices with dental status, where edentulous patients were reported to have higher MCI values.<sup>13,34,35</sup> We included only classes I and II according to Eichner in our study, in order to achieve reproducible horizontal and vertical dimension and to standardize the orthopantomographs more easily. It would have been fairly impossible to achieve reproducible positions of the skull and mandible with edentulous osseal specimens. Although there were no samples with extremely reduced occlusal support, we found significant correlation between inferior cortex appearance and dental status. This lack of occlusal support in premolar and molar regions results in reduced projection of occlusal forces on mandible with concomitant bone resorption and higher MCI values<sup>35</sup> MI, AI and GI were reported to reliably reflect systemic bone mass loss.<sup>18,20,23,30</sup> It is scientifically substantiated that bone mass loss due to osteoporosis affects also mandibular bones, and

that this can be detected in routine orthopantomograph or periapical radiograph examination by dentists. Moreover, it was recommended that patients having MI values  $<3$  mm be referred for bone densitometry tests for osteopenia/osteoporosis diagnosis.<sup>18</sup> Studies dealing with the assessment of critical bone thickness in mental region (MI) demonstrated that MI decreases with age, is lower in females than in males and is lower in patients with osteoporosis than in healthy individuals.<sup>17,34–36</sup> These differences were not observed in our sample.

Similarly, studies on cortical bone measurements in the antegonial region (AI) report on negative correlation between AI and age, lower AI in females and edentulous individuals than in dentate and denture-wearing individuals.<sup>13,17,34</sup> Our results agree only partially, in that the thickness of cortical bone at antegonion was greater in males unilaterally.

The decrease in cortical bone thickness in the gonial region (GI), particularly in females, was associated with osteoporosis.<sup>9,34</sup> The observed significantly lower GI in our study could not however be explained by osteoporosis because the sample consisted of the young individuals, but perhaps the stronger biting force in males could explain the difference.

In developing a method for densitometry of the mandible in the periapical X-rays, Nackaerts et al.<sup>37</sup> performed their measurements on intraoral digital X-rays recorded on cadavers. They used aluminium stepwedge for determining the grey scale and expressing the degree of decalcification, and with the help of specific software they obtained the reference scale for evaluating the bone mineral density on intraoral X-rays. Similarly, we could express bone mineral density as copper stepwedge equivalents, since we inserted the copper stepwedge in the cassette before the orthopantomographs recording. Anyway, the values could not be simply compared with those in the present populations without introducing a corrective index. We could therefore estimate the correlation between radiomorphometric indices and bone mineral densities in terms of copper stepwedge equivalents for a historic population from the 18th century. It should be expected that the correlation index would differ from the one in present populations because of the factors influencing X-ray based bone densitometry methods, including the lack of superimposed soft tissue, entrapped air in the bones, and changed mineral composition of bones. Even if we were to obtain this index, it would be hard to compare bone mineral densities with the values reported for the present populations at particular regions of interest, because the soil composition and conditions at which mineral salts deposition took place during a centuries long period cannot be fully reproduced.

From the radiomorphometric indices recorded in the 18th century population of Požega, we can conclude that they are comparable to the values reported for the present populations of Northern Croatia regions. In our sample, there were only two individuals with MI less than 3 mm which should be suspected for osteopenia. It is known that during the 18th century in Požega hygiene was pretty low, which together with humid surroundings due to numerous swamps, favoured big plague epidemic in 1739.<sup>33</sup> Anyway, only a limited number of conditions affect the skeleton, and plague cannot be identified by radiographic or morphologic analysis of bones.<sup>38</sup>

Finally, the assessment of mandibular radiomorphometric indices may be considered useful in estimating systemic bone tissue condition in historic populations. From the obtained results we cannot make any conclusions about generalized malnutrition or calcium, phosphorus and vitamin D deprivation in the historic population studied.

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## Competing interests

None declared.

## Ethical approval

Not required.

## Authors contributions

AIM analyzed and interpreted data and wrote manuscript, JM performed statistical analysis and generated Figs. 2 and 3, MV estimated age of samples and critically reviewed the manuscript, DKZ performed radiomorphometric assessment and helped analyze data, GPM and SJ conceived the study.

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