The association between linear enamel hypoplasia, cribra orbitalia and porotic hyperostosis in a South African skeletal sample

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Abstract

Background Linear enamel hypoplasia (LEH) and porous cranial lesions such as cribra orbitalia (CO) and porotic hyperostosis (PH) are considered nonspecific indicators of early childhood metabolic stress.

Objectives The study aims to describe the association of LEH with CO and PH in a sample of the Kirsten Skeletal Collection (KSC) representing a cohort in the Western Cape, South Africa. This will be used to determine if one or more of these lesions can be used as an indicator of adult mortality.

Sample and Methods Skulls (n = 412) of both sexes with known demographics from the KSC were macroscopically scored for LEH, CO and PH lesions using a ring light. This study was ethically approved by the Health Research and Ethics Committee of Stellenbosch University (S13/05/100). Statistical chi-square tests were used to determine the association between lesions and student’s t-tests to determine if there was a significant difference in mean age-of-death between groups.

Results There was no association when comparing LEH with CO and PH in the cohort, although an association was found between CO and PH. Individuals who exhibited the pathological lesions for LEH, CO or PH (or a combination thereof) were observed to have a younger mean age-at-death than the individuals without any of the three disease lesions.

Conclusions The lesions may potentially be used as an indicator of adult mortality. However, the results of this study might represent a biased population of low socio-economic status in the Western Cape as represented in the KSC, rather than the general population of the region.

Take-home message for students Pathological lesions observed on skeletal remains can provide valuable information to determine childhood physiological stresses and the standard of living of a population.
Introduction

Skeletal lesions on human remains are a direct product of interactions between biological, social and environmental factors. A number of skeletal indicators of metabolic disorders, nutritional deficiencies and hormonal disturbances with which to assess the nutritional status of a population, can be observed on bone (Ortner 2003). Metabolic disorders of bone are disorders in which a bone mass reduction results from insufficient osteoid production or excessive bone de-ossification (White et al. 2012). These disorders may be due to malnutrition, which may include inadequate intake of nutrients or insufficient absorption of ingested nutrients (Ortner 2003). During childhood and adolescence, the diploe of the cranial vault and medullary cavities of the long bones are the primary sites for erythropoiesis, and therefore juveniles are more likely to exhibit skeletal lesions associated with anaemia and other physiological stresses (Blom et al. 2005; Brickley 2018; Walker et al. 2009). Anaemic stress can cause an increase in erythrocyte production leading to hypertrophy and hyperplasia of the haemopoietic bone marrow and diploe (Mensforth et al. 1978). In long bones, anaemias are observed as metaphyseal widening and cortical thinning. However, in flat bones it presents as cranial bone thickening, coarsening of the trabeculae, and widening of the diploe.

Iron deficiency anaemia lesions such as cribra orbitalia (CO) are visible as pitting (small holes) in the outer layer of bone in the roof of the orbits (Brickley 2018; Ortner 2003; Walker et al. 2009). A number of factors have been implicated in causing CO, many of which are under debate, for example iron deficiency anaemia, sickle cell anaemia, malnutrition, scurvy, chronic gastrointestinal bleeding, ancylostomiasis and epidemic disease (Brickley 2018; Oxenham and Cavill 2010; Rivera and Mirazón Lahr 2017; Walker et al. 2009).

Porotic hyperostoses (PH) are lesions which manifest as areas of porosity on the outer table and diploe of the frontal, parietal, and occipital bones of the cranium accompanied by increased vault thickness (Brickley 2018; Merwe 2007; Walker et al. 2009). Although these lesions are similar to CO, the disease process is much more severe and is associated with major iron deficiency anaemia (Brickley 2018; Ortner 2003; Walker et al. 2009). In children, the bone can be thickened with large foramina, while in adults only remnants of the holes (frequently only pits) remain. Lesions seen in adults result from bone changes occurring in the growth period that have not undergone complete remodelling (Kozak and Krenz-Niedbała 2002; Merwe 2007; Stuart-Macadam 1987).

Dental enamel forms during juvenile growth and, in contrast to bone, never remodels after its formation. When dental enamel formation is disrupted, resulting in a reduction of enamel thickness (Goepferd and Flaitz 1981) due to metabolic insult, defects can occur in the tooth enamel structure (White et al. 2012). Linear enamel hypoplasia (LEH), also referred to as dental enamel hypoplasia or simply as dental hypoplasia, is a nonspecific quantitative enamel defect, presenting as three main forms of defects on teeth, as classified by Lukacs (1989): a) linear horizontal grooves, b) linear/non-linear pits, and c) linear grooves and pits in the tooth crown enamel as a result of defects in ameloblastic activity. This dental defect represents a short period when growth slowed down or stopped during the formation of the tooth crown (the foetal period) until the age of eruption of the last tooth (Goodman and Rose 1990; Steckel and Rose 2002). Robinson et al. (1983) have stated that the exact aetiologia of LEH cannot be specified, but likely factors that may result in LEH are
periapical inflammation or trauma to a deciduous tooth, fever, disease, nutritional deficiencies endocrine dysfunction, and generalised infection during odontogenesis. Due to these varied aetiological causes, LEH is considered a nonspecific indicator of human population health (Goodman and Armelagos 1988; Huss-Ashmore et al. 1982).

The aim of this study is to describe the association of LEH with CO and PH in the Kirsten Skeletal Collection (KSC) and thereby to determine if one or more of these lesions can be used as an indicator of adult mortality.

### Sample & Methods

#### Sample

The Kirsten Skeletal Collection (KSC) is a registered skeletal biobank (B20/09/004) housed in the Division of Clinical Anatomy, Faculty of Medicine and Health Sciences at Stellenbosch University in South Africa. The KSC contains skeletal specimens obtained mainly from cadavers with known records, used for the training of medical students at the University (Labuschagne and Mathey 2000; Alblas et al. 2018). Under the Human Tissue Act 65 (South African National Department of Health 1983), the more recent National Health Act 61 (South African National Department of Health 2004) and the protection of the regional Inspector of Anatomy, Stellenbosch University is allowed to receive cadavers for both teaching and research purposes. The KSC represents a population who lived between the mid- and late 20th century, as most of the individuals were born between 1920 and 1949 (42%) and died between 1970 and 1989 (54%) (Alblas et al. 2018; Pfeiffer et al. 2016). The KSC has been reported to have an overrepresentation of males, aged individuals, and people with lower socio-economic status (Alblas et al. 2018).

This study was ethically approved by the Health Research and Ethics Committee (HREC) of Stellenbosch University (S13/05/100). The skeletal remains of 412 individuals from the KSC, were analysed for the purpose of this study. Individuals were chosen according to their completeness, and those with severe damage or trauma to the crania were excluded from the sample. Skeletons with known cadaver records with information concerning their sex, age-at-death, population affinity, and cause of death were used for analysis. Cause of death and general frailty of the skeleton were taken into account when collecting and processing the data, although both factors were not used as predictors (of mortality) in this study.

#### Methods

According to Goodman et al. (1988), the two maxillary incisors and the two mandibular canines are to be used for scoring the presence of LEH in the teeth. However, in this study, many individuals did not have incisors to analyse. Modification and extraction of partial or full dentition were normal for inhabitants of the Western Cape during the 20th century (Alblas et al. 2018; Friedling and Morris 2007). Therefore, all available dental elements on the maxillae and mandibles were macroscopically assessed for the presence of LEH. A dental probe was used to manually detect any sign of growth-arrest lines or pitting in the crown enamel of a tooth. Enamel hypoplasia was marked as ‘Present’ if at least one defect in the tooth enamel (horizontal lines or pitting) was visible, whilst teeth showing no defects were scored as ‘Absent’. No attempt
was made to record the position of the lesion on the surface of the crown, and the number of lines per tooth was not counted. Porotic hyperostosis was observed as porosity on the external cranial vault and CO as porosity in the orbital roof (Facchini et al. 2004; Walker et al. 2009). Any sign of pitting with accompanying vault thickness on the frontal, parietal and/or occipital surfaces was scored positively for each individual (Stuart-Macadam 1985), and an expanded diploe was also considered. So, the lesions were scored as ‘Present’ in the individual for all severity grades of PH and CO outlined in the grading system of Buikstra and Ubelaker (1994). Post-mortem damage due to storage erosion and handling of artefacts were considered when the lesions were analysed, with the use of a magnifying ring light.

**Results**

The sample consisted of 412 individuals ranging from 12 to 101 years of age. As illustrated in Figure 1A, the age-at-death was normally distributed across the total sample. The proportion of males in the sample was noticeably larger compared to females, with the male-to-female ratio calculated at 2.3:1 (Figure 1B). Examples of LEH, CO and PH in the sample can be seen in Figure 2. The frequency distribution and statistical analysis of LEH with sex, CO and PH is summarised in Table 1. Figure 3 illustrates the frequency of males and females with LEH in co-occurrence with CO and PH. Enamel hypoplasia was observed in a total of 35 (8.5%) of the total 412 crania examined (Table 2). Within the sample, 34 of the 69 individuals with PH did not present with CO, whereas 25 of the 60 individuals with CO did not show signs of PH. Regarding the age-at-death analyses, it was noted that the average age recorded for individuals with LEH was generally lower
Figure 1 Frequency distribution of age-at-death in the sample displayed a normality curve. A) Normal distribution of ages across total sample (ungrouped) and B) within male and females (grouped). Figures are generated in RStudio (R Core Team 2022).

compared to those who did not present with LEH (Table 3). When considering sex as a variable in addition to LEH presence, both males and females presenting with LEH died at a younger age. For males, the mean age-at-death was calculated at 9.79 years younger for those with LEH, whilst females died approximately 13 years younger when showing signs of LEH (Table 3). The differences in mean age-at-death within male and female groups showing signs of LEH were significant (t-test: p < 0.05) and

Figure 2 Visual depiction of the disease lesions observed in the sample. A) Linear enamel hypoplasia on left mandibular incisors and canine, B) cribra orbitalia in the left orbital roof, and C) porotic hyperostosis on left parietal bone. Photographs by Chantelle Marais, 2022.
is illustrated with the use of boxplots in Figure 4.

After grouping the sample according to LEH presence/absence, it was noted that individuals with both LEH and CO died at a mean age of 40.11 ± 15.14 years (Table 3). Individuals with LEH but no CO died at an average age of 39.23 ± 14.25 years (Table 3). This difference between the mean ages had a p-value of 0.88 and was therefore not significant (Figure 5). Meanwhile, within the ‘LEH Absent’ group, individuals with CO died at a younger mean age (46.90 ±11.93 years) than those who showed no signs of either LEH or CO (51.65 ± 15.64 years). The difference between these mean ages was found to be significant (p = 0.014) (Figure 5).

A similar trend was observed with the age-at-death analyses when considering LEH presence in addition to the prevalence of PH. Individuals with LEH but not PH experienced a slightly longer lifespan of 40.60 ± 14.62 years compared to the 32.60 ± 10.45 years for individuals presenting with both LEH and PH lesions (Table 3), although this finding was not significant (p = 0.18) (Figure 6). A significant difference (p < 0.05) between the average age-at-death for those with or without PH was observed within the ‘LEH Absent’ group (Figure 6).

**Discussion**

The study aimed to describe the association of linear enamel hypoplasia (LEH) with cribra orbitalia (CO) and porotic hyperostosis (PH) in the Kirsten Skeletal
Collection (KSC) and thereby determine if one or more of these lesions can be used as an indicator of adult mortality.

**Prevalence and association of early physiological stress markings in the KSC**

LEH is a condition that follows a disruption of ameloblastic function during odontogenesis, resulting in a reduction of enamel thickness (Goepferd and Flaitz 1981) and is considered a nonspecific indicator of human population health (Goodman and Armelagos 1988; Huss-Ashmore et al. 1982).

In population-specific studies, the prevalence of LEH in different populations varies between 3.0% (Seow et al. 1987) in developed countries or well-nourished populations, to 99.0% (Pascoe and Seow 1994) in developing countries or populations living under low socio-economic conditions (Goodman et al. 1980; Malville 1997; Saunders and Keenleyside 1999). Genetic factors, as well as exposure to similar environmental stresses, can have an influence on the susceptibility of LEH. For example,

<table>
<thead>
<tr>
<th>Linear enamel hypoplasia</th>
<th>P-value</th>
<th>Effect size (Phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>266 (64.6%)</td>
<td>22 (5.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>111 (26.9%)</td>
<td>13 (3.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>377 (91.5%)</td>
<td>35 (8.5%)</td>
</tr>
</tbody>
</table>

Table 1 Frequency distribution and statistical analysis of Linear Enamel Hypoplasia, with Sex, Cribia Orbitalia and Porotic Hyperostosis variables in the Kirsten Skeletal Collection (KSC), Stellenbosch, South Africa.

<table>
<thead>
<tr>
<th>Cribra orbitalia</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>326 (79.1%)</td>
<td>26 (6.3%)</td>
</tr>
<tr>
<td>Present</td>
<td>51 (12.4%)</td>
<td>9 (2.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>377 (91.5%)</td>
<td>35 (8.5%)</td>
</tr>
</tbody>
</table>

Table 2 Contingency table showing the frequencies and associations of Cribia Orbitalia and Porotic Hyperostosis in the Kirsten Skeletal Collection (KSC), Stellenbosch, South Africa.

<table>
<thead>
<tr>
<th>Porotic hyperostosis</th>
<th>Pearson’s chi-sq</th>
<th>Effect size (Phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>318 (77.18%)</td>
<td>34 (8.25%)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (6.07%)</td>
<td>35 (8.50%)</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>69</td>
</tr>
</tbody>
</table>
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Figure 4 Boxplots showing the distribution of age-at-death in males and females with LEH. The differences in average age-at-death for individuals with or without LEH was determined by using a Student’s t-test. Figures are generated in RStudio (R Core Team 2022).

A study on young Australian Aboriginal children from Bathurst Island (Pascoe and Seow 1994), showed a prevalence of 99.0% LEH, with nearly all the patients presenting with a full range of medical problems from similar environmental stressors. A

Table 3 Mean, median and standard deviation for adult age-at-death in Linear Enamel Hypoplasia (LEH) by other variables such as sex, Cribia Orbitalia (CO), and Porotic Hyperostosis (PH) in the Kirsten Skeletal Collection (KSC), Stellenbosch, South Africa.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEH Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PH Absent</td>
<td>313</td>
<td>52.22</td>
<td>51</td>
<td>15.55</td>
</tr>
<tr>
<td>PH Present</td>
<td>64</td>
<td>45.09</td>
<td>45.5</td>
<td>12.21</td>
</tr>
<tr>
<td>LEH Present</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PH Absent</td>
<td>30</td>
<td>40.60</td>
<td>41</td>
<td>14.62</td>
</tr>
<tr>
<td>PH Present</td>
<td>5</td>
<td>32.60</td>
<td>33</td>
<td>10.45</td>
</tr>
<tr>
<td>LEH Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO Absent</td>
<td>326</td>
<td>51.65</td>
<td>50</td>
<td>15.64</td>
</tr>
<tr>
<td>CO Present</td>
<td>51</td>
<td>46.90</td>
<td>45</td>
<td>11.93</td>
</tr>
<tr>
<td>LEH Present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO Absent</td>
<td>26</td>
<td>39.23</td>
<td>41</td>
<td>14.25</td>
</tr>
<tr>
<td>CO Present</td>
<td>9</td>
<td>40.11</td>
<td>34</td>
<td>15.14</td>
</tr>
<tr>
<td>LEH Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>266</td>
<td>52.38</td>
<td>51</td>
<td>14.99</td>
</tr>
<tr>
<td>Female</td>
<td>111</td>
<td>47.74</td>
<td>47</td>
<td>15.48</td>
</tr>
<tr>
<td>LEH Present</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>42.59</td>
<td>41.5</td>
<td>12.20</td>
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<tr>
<td>Female</td>
<td>13</td>
<td>34.15</td>
<td>28.0</td>
<td>16.34</td>
</tr>
</tbody>
</table>
similar study on Guatemalan children, however, showed a significantly higher prevalence of LEH among siblings, while the general prevalence of LEH in the population fell between 18.0% and 24.0% (Infante and Gillespie 1974), in this instance suggesting a genetic influence.

The prevalence of LEH in the present study was recorded in 35 (8.5%) of the 412 individuals analysed. The KSC comprises of individuals who lived between the mid- and late 20th century with low socio-economic conditions and poor access to health care (Labuschagne and Mathey 2000; Pfeiffer et al. 2016). These communities also showed a high prevalence of pulmonary infectious diseases, such as pneumonia and tuberculosis (Geldenhuys 2014), and a high prevalence of infectious diseases (Alblas et al. 2018) that may contribute to the formation of LEH (Aufderheide and Rodriguez-Martín 1998). Therefore, a higher number of LEH was expected. The low prevalence of LEH observed in the current sample may be due to the lack of dentition available for analysis and the result is not a true representation of the signs of LEH in the individuals in the KSC.

The results of this study showed no statistical significance between LEH presence and sex; however, the prevalence in males is slightly higher than in females. Most studies comparing sexes showed no difference in the prevalence of LEH (Infante and...
Gillespie 1974; Kozak and Krenz-Niedbala 2002; Malville 1997). Studies conducted by Li et al. (1995) as well as Saunders and Keenleyside (1999) showed a statistically higher prevalence of LEH in males than in females, supporting the theory that males are more sensitive to external environmental effects during growth than females. In a review by Stinson (1985) on sex differences to environmental sensitivity, it was concluded that this theory is difficult to prove in the postnatal period due to complex environmental differences and cultural differences between population groups. Prenatal differences in environmental sensitivity show more consistent trends with males showing a higher mortality rate than females prenatally (Stinson 1985). The same study also showed that maternal nutritional supplementation improved growth in males more than in females (Stinson 1985).

The current study did not show a significant association between the presence of LEH with the presence of either CO or PH. CO and PH can occur both during adult- and childhood, although the lesions (especially CO) are more apparent in children (Walker et al. 2009). Lesions of CO and PH are believed to be related. Previous authors have suggested that bony lesions usually start in the orbits and then if metabolic stress continues, spread to the parietal and occipital bones due to
a difference in diameter and microstructure of the bones (Facchini et al. 2004; Stuart-Macadam 1985; Wapler et al. 2004). Anaemic stress can cause an increase in erythrocyte production leading to hypertrophy and hyperplasia of the haemopoietic bone marrow and diploe (Mensforth et al. 1978). Porotic hyperostosis is usually associated with CO while CO frequently occurs without PH (Lallo et al. 1977). The present study found that 35 of the 412 individuals (8.5%) showed signs of both CO and PH. Of the total sample, 34 of the 69 individuals with PH did not present with CO, whereas 25 of the 60 individuals with CO did not show signs of PH. The results for the correlation analysis between CO and PH was significant.

In most studies, females showed a higher prevalence of signs of anaemia (collectively seen as CO and PH) than males (Bharati and Basu 1990; Kozak and Krenz-Niedbała 2002; Wapler et al. 2004; Webb 1982). The reason for this lies in the physiology of a female who, during, for example, menstruation, childbirth, and lactation loses iron (Kozak and Krenz-Niedbała 2002). Rosso and Lederman (1982) suggested that developing countries, like South Africa, have an anaemic bias towards young females due to a tendency for pregnancies at a young age, malnutrition, and poor access to healthcare facilities. Furthermore, males have a larger iron storage ability than females due to slower growth in late adolescence, thereby improving their iron level status, thus females are more likely to exhibit signs of iron deficiency anaemia than males (typically). This contradicts the findings of the present study which showed a higher prevalence of CO and PH in males than in females, although this finding was not significant. Only a few other studies found a higher prevalence in males but none of them with significant differences (Facchini et al. 2004; Fairgrieve and Molto 2000). This suggests that a factor other than anaemia may have contributed to CO and PH. Other factors previously suggested include post-mortem erosion, hyper-vascularisation, osteoporosis and osteitis (Wapler et al. 2004).

Age-at-death analysis

It has been hypothesised that individuals with more stress episodes during childhood are more prone to higher morbidity and early mortality. In an attempt to understand whether these early physiological stress markers can provide insight to the expected adult mortality in the KSC, the age-at-death of the individuals who exhibited any one of the three lesions (LEH, CO or PH) were compared in terms of their average age to all the individuals who did not exhibit one of the three pathological lesions.

In general, the individuals in this study who exhibited the pathological lesions for LEH, CO or PH had a younger mean age-at-death than the individuals without any of the three lesions. This stands in contrast to results from previous studies in which the authors did not find any correlation between age-at-death and LEH (Saunders and Keenleyside 1999). A possible explanation for the decrease in LEH may be that the children developing this defect were exposed to infections and malnutrition not just during childhood but throughout life resulting in death at an earlier age. The individuals without the defect were most likely better nourished and lived under better socio-economic conditions, thereby decreasing the opportunity of obtaining infectious diseases to a similar extent.

The results of the current study showed that LEH, PH and CO may be used (separately) as an indicator for adult age-at-death, but not necessarily in combination. One should also note that porous lesions like CO, often have a more complicated
aetiology, such as subperiosteal reactions associated with nutritional deficiencies, and complicated by a high alcohol intake (Mensforth et al. 1978; Steckel and Rose 2002). Non-specific infectious diseases, among other causes, play a more prominent role in the aetiology of subperiosteal bone reactions, which can occur throughout life (Brickley 2018; Ortner 2003). Due to the lack of access to medical records of the individuals in this sample, the usefulness of the disease lesions as indicators of a healthy or diseased society are not exact and cannot be referenced to the social context of the society. In light of the Osteological Paradox (Wood et al. 1992), these disease lesions’ interpretations can be twofold. On one side, they might signify a population which endured numerous health challenges, portraying a frail demographic. On the contrary, they could represent a resilient group that endured health problems long enough for the lesions to manifest on the skull.

Further speculation as to possible specific causes of the observed lesions of CO and PH within the sample was not attempted, as the severity and state (active or healed) of the lesions were not taken into account during data collection. Other disease conditions observable from the skeletal remains were also excluded from the current study and could thus be useful to include in future studies.

Individuals who exhibited the pathological lesions for LEH, CO, or PH (or a combination thereof) were observed to have a younger mean age-at-death than the individuals without any of the three lesions. Thus, the lesions may potentially be used as an indicator of adult mortality. The results of this study might represent a biased population of low socio-economic status in the Western Cape, rather than the general population of the region (Labuschagne and Mathey 2000; Pfeiffer et al. 2016).

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