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THE IMPACT OF ENVIRONMENTAL STRESS ON MINOR CONGENITAL DEFECTS: MEASURING THE ASSOCIATION BETWEEN MCD, STRESS MARKERS AND BONE LENGTH

ABSTRACT: A sample of 117 adult individuals from the Lisbon Identified Skeletal Collection (aka Luís Lopes Collection) was used to test the association between six minor congenital defects (MCD) and stress markers. The goal was to assess if any of the tested MCD might be considered a useful indicator of stress in early life. The variables included in the study are manubrium mesosternal joint fusion, sternum hyperplasia, sternal aperture, sternal caudal clefting, notochord defects and hypoglossal canal, cribra orbitalia*, vertebral neural canal size, femur and tibia maximum length. Sternal caudal clefting has a statistically significant correlation with femur length, in males. The direction of the correlation indicates that males with sternal caudal clefting have longer femurs. Sternum hyperplasia is correlated with anteroposterior and transverse diameters of thoracic vertebrae, for males. Males with sternum hyperplasia have narrower AP diameters and wider TR diameters. Double hypoglossal canal is correlated with TR diameters of thoracic vertebrae, in females. Females with double hypoglossal canal have narrower TR diameters of thoracic vertebrae. Manubrium mesosternal joint fusion, sternal aperture, notochord defects and sternal caudal clefting were not associated with any of the tested variables. Both sternum hyperplasia and double hypoglossal canal might have potential to capture environmental stress, in utero, but more studies are required to confirm this result in other collections.*

KEY WORDS: Minor congenital defects - Stress markers - Identified skeletal collections

INTRODUCTION

Environmental stress (e.g. infectious diseases, malnutrition or water-borne parasites) leaves permanent marks in the skeletal, usually called non-specific stress markers. Stress, in this context, can be defined as a disruption in homeostasis in developing or growing process of a particular individual with permanent

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physical outcomes. Tough environmental stress might come in several different forms, the skeleton has few ways to record it.

Osteoarchaeological research made interesting approaches to the study of MCD, in order to test its environmental etiology. Bergman (1993) found an association between *cribra orbitalia* and several MCD. Sture (2001) analyzed several MCD of axial skeletal in Medieval urban and rural English populations, – one of the sites dating from middle of tenth to late twelfth centuries, and the other three from early twelfth century up to sixteenth century –, to test a possible association between MCD and settlement type, reasoning that higher population density and urban lifestyle would create a harsher environment in urban populations resulting in higher prevalence rates of MCD. In fact, from five English Medieval populations, only one failed to demonstrate a positive association between urban settlements and higher MCD. Also, individuals with MCD were more likely to exhibit stress markers (*cribra orbitalia*, enamel hypoplasias and tibia periostitis). Later on, Tancock (2014) failed to test the association between MCD and settlement type in a study of Northeast England, from eighteenth and nineteenth centuries, but argued towards the possibility of similar harsh living conditions in both type of settlements. Amoroso (2020) failed to found an association between several MCD of sternum and vertebral column and age-at-death.

In nineteenth and early twentieth century, Lisbon living conditions were hard for most of the population, but particularly to more vulnerable individuals, like pregnant women and small children (Veiga, 2004). Exposure to several stressors, since embryogenesis up to late adolescence, might trigger the development of skeletal stress markers, including Minor Congenital Defects (MCD), *cribra orbitalia* and stunted VNC size, femur and tibia length, which can be used as a proxy for childhood environmental conditions. The presence of several stress markers in the same individual, formed in different periods of life course, represent a long-lasting exposure to stressful events during development and growth (Goodman *et al.* 1988).

Minor congenital defects

Minor congenital defects (MCD) are abnormal variants of bones, originated by an injurious effect of an extrinsic (i.e. environmental) or intrinsic (i.e. genetic) factor on the embryo (Webster, Wreede 2012). MCD develop in three ways: (a) whenever there is an abnormal genotype, the defect is expressed even in normal environment; (b) whereas, environmental negative stimuli hamper development of normal genotype; (c) a gene-environment interaction occurs, influencing the degree of the defect manifestation (Carlson 2009). Most features in humans are both biological (genetic) and cultural (environmental) such as sex (as proxy for gender), age or stature. MCD may be another example (Saunders, Rainey 2008, Sture 2001, Tancock 2014). Embryogenesis, which occurs between weeks 3 and 8 (Carlson 2009), is the most critical period for developing MCD.

MCD have different designations in the literature, with slightly different meanings: developmental field defects (Barnes 1994); congenital defects (e.g. Aufderheide, Rodríguez-Martín 1998); non-metric(al) or discrete traits (Saunders, Rainney 2008); minor skeletal variants (e.g. Trinkaus 1978); epigenetic variants (e.g. Hauser, de Stefano 1989); and atavism (Ossenberg 1969). The term Minor Congenital Defects is going to be used in this study.

A vast research on MCD, is dedicated to biological distance between and within populations, assuming most of these traits are under genetic control. Population differences in prevalence of these traits are explained by genetic drift (Barnes 2012). However, since early 1950's there are evidences corroborating the existence of an environmental component (e.g. nutritional deficiency) in the formation of many MCD in animals (Gruneberg 1952). See *et al.* (2008) in a study about vitamin A deficiency on pregnant rats found fetus had, among other minor anomalies, basioccipital and sternum malformations. Therefore, it might be infered vitamin A deficiency and other nutritional deficits in the pregnant human female might be responsible for some MCD.

Cribra orbitalia

Cribra orbitalia is a pathological condition characterized by porotic structures in orbital roof (Ortner 2003). Although, probably not specific of a particular disease, *cribra orbitalia* is widely used as a non-specific stress marker in human past populations, including in Portuguese medieval and modern samples (Temple 2010, Watts 2013, Garcia 2007, Hens *et al.* 2019). Commonly, *cribra orbitalia* was connected with similar porotic modifications in cranial vault (porotic hyperostosis) and both were considered to be caused by iron-deficiency anemia (e.g. Carlson *et al.* 1974, El-Najjar *et al.* 1976). Iron-deficiency anemia hypothesis has been challenged by Walker *et al.* (2009) claiming this hypothesis is not consistent with late hematological

research that shows iron-deficiency alone is not capable of producing these lesions. Instead, Walker *et al.* (2009) defended that both porotic hyperostosis and many types of *cribra orbitalia* are consequence of vitamins C and B12 deficiency. However, Oxenham and Cavill (2010) consider iron-deficiency anemia a still suitable cause to *cribra orbitalia*, although exact cause for porotic lesions remains puzzling, due to limited bone response to pathologies (McIlvaine 2015). In sum, iron-deficiency anemia may be caused by starvation diets but also by high pathogen load, acute or chronic blood loss or water-borne parasites (Stuart-Macadam 2006, Hens *et al.* 2019).

Vertebral neural canal

Although vertebral body longitudinal growth is processed during the entire course of individual growth, most neural arch reaches their full size during early childhood (Newman, Gowland 2015). Fusion of spinous process takes place between first and second year of life, followed by fusion of neural arch to vertebral by, between 3 and 5 years old (Scheuer, Black 2000). Anteroposterior (AP) and transverse diameters (TR) attain 95% of complete growth at 5 years old (Diméglio 1993) and at 6 years old, neurocentral junctions are fused (Scheuer, Black 2000). AP neural canal diameter starts to grow in utero, at 6th week, attains 70% of complete growth at birth (Ursu *et al.* 1996) and reaches full size at approximately 4 years old (Papp *et al.* 1994). TR neural canal diameter development begins in utero and can grow until 15 to 17 years old (Newman, Gowland 2015). Lumbar vertebrae have a longer growth period than cervical and thoracic vertebrae (Clark *et al.* 1986).

Besides possible genetic influence on vertebral neural canal (VNC) size, several environmental stressors (e.g. maternal illness, toxins, infections, placental-insufficiency), pre- and post-natal, might result in permanent stunt growth (Papp *et al.* 1994). Therefore, AP and TR diameters can be used as a non-specific stress marker, capturing stressful events of infancy and early childhood (AP) and later childhood and adolescence (TR). VNC has been used as a stress marker in several studies, where VNC size (for several AP and TR diameters of cervical, thoracic and lumbar vertebrae) were positively statistically significantly associated with age-at-death (Amoroso, Garcia 2018, Clark *et al.* 1986, Watts 2011, 2015), and with academic status (Porter *et al.* 1987) and negatively statistically significantly associated with lower-back pain and trauma (Porter *et al.* 1987).

Femur and tibia length

Femur and tibia are the fastest-growing bones in the body, which is believe to make them the most sensitive bones to environmental insults (Eveleth, Tanner 1990). Femur growth starts during $7^{th}/8^{th}$ week of embryonic development until up to 18–20 years of age, when all epiphyses fuse (Tanner 1989, Scheuer, Black 2000). Tibia starts developing a few days after the femur, around week 8 of the embryo (Ferretti, Tickle 1997) and ends growing up to 17 (females) and 19 (males) years old, when proximal epiphyses fuses (Scheuer, Black 2000).

Long bones length is under both genetic and environmental control (Tanner 1989, King, Ulijaszek 1999). Hirschhorn *et al.* (2001) suggests that around 75–90% of human height is under genetic influence. Thus, several environmental factors can constraint growth, resulting in stunting (low height-for-age) (Larsen 2002) or prompt a positive secular trend consistent with a general improvement in standard living conditions of the population (Padez 2002). Femur length constraints can start in uterus; there are several clinical studies testifying that a short femur length is one of the characteristics of a small-for-gestational age fetus (Todros *et al.* 1996, Zalel *et al.* 2002). Both femur and tibia have potential to catch-up growth, hindering the association between their final length and hazard conditions early in life, but both are still widely used in bioarcheology as a proxy for childhood environment (Cardoso, Garcia 2009, Ives, Humphrey 2017, Mays *et al.* 2008, Wasterlain *et al.* 2018). Numerous studies show that the tibia is more susceptible to environmental stress than the femur, resulting in greater variation in tibia length, either within (Holliday, Ruff 2001) or between populations (Meadows, Jantz 1995, 1999). This differentiated response to environmental insults is the rational for using both lower bones in the analysis.

Research rationale

For a MCD to be considered a useful stress marker in biological research, it should be correlated with other stress indicators. It will be tested if individuals who went through stressful events during embryogenesis resulting in MCD, have a decreased buffering ability against environmental stress during growth and therefore are predisposed to develop iron-deficiency anemia in early infancy (showed as *cribra orbitalia*) and growth faltering (manifested in small VNC and short femur and tibia). Additionally, having skeletal elements that comprises the entire growth

period, life course and accumulation concepts can be tested. *Cribra orbitalia* is to test the epidemiological concept of accumulation that states stressful events, occurring early in life tend to be replicated (Blane 2006). The use of VNC size, femur and tibia's length is justifiable because all bones can have their growth stunted in utero due to environmental stress (although they have the potential to catch-up their growth trajectory).

MATERIAL AND METHODS

The Lisbon Identified Skeletal Collection, deposited at MUHNAC, Lisbon, Portugal, consists in approximately 750 individuals of known sex and age-at-death, who died in Lisbon, between 1881 and 1970. The sample consists of 117 adult individuals – with fused femur and tibia epiphyses –, randomly chosen, from 16 to 92 years old, from which 54.7% (n=64) are females and 45.3% (n=53) are males (*Table 1*). Individuals were born between 1836 and 1935 and died between 1896 and 1961.

MCD were chosen according to the following criteria: a) show no alterations during life span; b) can be easily distinguishable from skeletal markers related to activity patterns or artificial modifications; c) are not life threatening; and d) have expected high prevalence rates, according to a previous study on the same collection (Amoroso 2020). MCD were recorded, following Barnes (2012): manubrium mesosternal joint fusion (*Figure 1*), sternum hyperplasia (*Figure 2*), sternal aperture (*Figure 3*), sternal caudal clefting (*Figure 4*), notochord defects (*Figure 5*) and hypoglossal canal (*Figure 6*) (*Table 2*).

TABLE 1: The sample, by age group and sex.

Age group/Sex	Females	Males	Total
16–19	6	3	9
20–29	8	6	14
30–39	7	9	16
40–49	9	7	16
50–59	9	8	17
60–69	10	6	16
70–79	8	5	13
≥ 80	7	9	16
Total	64	53	117

FIGURE 1: Manubrium mesosternal joint fusion. MB61-01178 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph A. Amoroso, courtesy University of Lisbon).

FIGURE 2: Sternum hyperplasia. MB61-01621 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph A. Amoroso, courtesy University of Lisbon).

FIGURE 3: Sternal aperture. MB61-01624 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph A. Amoroso, courtesy University of Lisbon).

FIGURE 4: Sternal caudal clefting. MB61-01593 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph A. Amoroso, courtesy University of Lisbon).

FIGURE 5: Notochord defect. MB61-00517 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph A. Amoroso, courtesy University of Lisbon).

FIGURE 6: Hypoglossal canal. MB61-00353 Luis Lopes Collections, MUHNAC, University of Lisbon (photograph S. Garcia, courtesy University of Lisbon).

TABLE 2: Minor congenital defects recorded, according to Barnes (2012).

Age group/Sex	Females	Males	Total
16–19	6	3	9
20–29	8	6	14
30–39	7	9	16
40–49	9	7	16
50–59	9	8	17
60–69	10	6	16
70–79	8	5	13
≥ 80	7	9	16
Total	64	53	117

A xiphoid process longer than 5 cm (Anderson *et al.* 2020), or more than half the size of mesosternal size, was considered extra-long. *Cribra orbitalia* (porous lesions on the orbital roof) was recorded as present/absent, with at least one observable orbit. Anteroposterior (AP) and transverse (TR) diameters of each VNC were measured only in complete vertebral segments. AP diameter was measured from posterior portion of vertebral body to the utmost opposite point of neural canal anterior to spinous process. TR diameter was measured as the longest distance between medial surfaces of left and right pedicles. Both measurements were made from superior view of vertebrae (Watts 2011). Maximum femur and tibia length were measured on the left bone, except in cases of poor preservation or pathology (fractures and knee osteoarthritis) (Buikstra, Ubelaker 1994).

Crude prevalence of MCD and *cribra orbitalia* was calculated by dividing the number of defects by the number of observed individuals. Fisher's exact test was used to identify sex differences in MCD and *cribra orbitalia*. An independent sample t-test was used to test sex differences in VNC diameters, femur and tibia length. Fisher's exact test was also used to test if there was any relationship between MCD and *cribra orbitalia*. A point-biserial correlation was used to measure strength and direction of the association between MCD and VNC diameters, femur and tibia length. To test life course concept, individuals where assigned to different groups, according to the timing of the stress markers exhibited: a) no stress markers; b) with MCD (only the ones for which was found a correlation with another variable); c) with *cribra orbitalia* or stunted AP diameter of VNC size; d) stunted TR diameters of VNC size or stunted femur or stunted tibia; and e) at least two stress markers assigned in two different groups. VNC diameters and femur and tibia length were considered stunted when their measure was less than the mean measure minus two standard deviations. A one-way ANOVA was used to test if there were any differences in age-at-death between groups.

All statistical analyses were done using IBM SPSS Version 25.

To test measurement reliability and replicability, the first author re-measured 393 diameters and the second author re-measured 434 diameters. Technical error of measurement (TEM) was calculated according to Ulijaszek and Kerr (1999), using the following equation:

$$\sqrt{\Sigma \frac{D^2}{2N}}$$

, where D is the difference between both measurements and N is the total number of measurements. Reliability coefficient was then calculated using the following equation:

$$\frac{TEM^2}{SD^2}$$

, where SD is the variance (inter-subject variance) for each set of measurements (Ulijaszek, Kerr 1999).

RESULTS

Crude prevalence of MCD and *cribra orbitalia*, mean size and standard deviation of VNC diameters, femur and tibia length, and sex differences for all variables is presented in *Table 3*. All VNC diameters have statistically significant sex differences, with males having larger diameters than females, except for lumbar AP diameters with no sex differences. Male's femur and tibia length are also statistically significantly larger than females'. *Cribra orbitalia* and MCD have no statistically significant sex differences.

None of MCD analyzed is associated with *cribra orbitalia*. Femur length, for males, is statistically significant correlated with sternal caudal clefting, albeit weak (r=0.384; p=0.012); males with sternal caudal clefting have longer femur. Tibia length, for females, is statistically significant correlated with notochord defects, though weak (r=0.318; p=0.013); females with notochord defects have longer tibia.

TABLE 3: Crude prevalence of MCD and *cribra orbitalia*; mean size and standard deviation of VNC diameters, femur and tibia length and sex differences.

	FEMALES			MALES			TOTAL			Sex diff.
	Mean	Std	Prev.	Mean	Std	Prev.	Mean	Std	Prev.	
Cervical AP	16.55	1.06	-	17.84	1.12	-	17.06	1.25	-	p<0.001
Cervical TR	23.56	1.08	-	24.50	1.09	-	23.92	1.17	-	p<0.001
Thoracic AP	15.51	1.10	-	16.21	1.83	-	15.87	1.14	-	p=0.006
Thoracic TR	16.47	1.15	-	17.17	1.35	-	16.83	1.30	-	p=0.016
Lumbar AP	16.72	1.60	-	16.68	1.82	-	16.70	1.70	-	p=0.902
Lumbar TR	22.07	2.06	-	22.95	1.80	-	22.48	1.99	-	p=0.021
Femur length	404.26	19.17	-	445.49	22.34	-	422.94	29.12	-	p<0.001
Tibia length	334.57	16.23	-	369.48	18.33		350.78	24.50	-	p<0.001
Cribra orbitalia	-	-	32.30	-	-	32.00	-	-	32.10	p=1.000
Manubrium mesosternal joint fusion	-	-	25.00	-	-	19.50	-	-	22.60	p=0.621
Sternum hyperplasia	-	-	11.50	-	-	7.10	-	-	9.60	p=0.727
Sternal aperture	-	-	11.50	-	-	14.30	-	-	12.80	p=0.762
Sternal caudal clefting	-	-	15.40	-	-	11.90	-	-	13.80	p=0.767
Pectus excavatum	-	-	26.90	-	-	14.60	-	-	21.50	p=0.205
Pectus carinatum	-	-	9.60	-	-	0.00	-	-	5.40	p=0.064
Notochord defects	-	-	21.90	-	-	13.20	-	-	17.90	p=0.333
Hypoglossal canal	-	-	17.20	-	-	34.00	-	-	24.80	p=0.052

Sternum hyperplasia is statistically significant, yet weakly correlated with male thoracic AP diameter (r=-0.360; p=0.040); males with sternum hyperplasia have narrower thoracic AP diameter. Sternum hyperplasia is statistically significant, though weakly correlated with male thoracic TR diameter (r=-0.347; p=0.048); males with sternum hyperplasia have wider thoracic TR diameter. Double hypoglossal canal is statistically significant, but weakly correlated with female thoracic TR diameter (r=-0.362; p=0.025); females with double hypoglossal canal have narrower thoracic TR diameter (Table 4). The ANOVA showed no statistically significant differences between groups, according to the timing of formation of their stress markers (females: p=0.735; males: p=0.125).

DISCUSSION

This study tested possible association between MCD and *cribra orbitalia*, VNC size, and femur and tibia length, in an Identified Skeletal Collection, from Portugal. Manubrium mesosternal joint fusion and sternal aperture failed to show a statistically significant association with femur and tibia length, *cribra orbitalia* and VNC size, therefore nothing indicates the utility of this MCD's as stress markers. Possibly because these three defects are under stronger genetic control, having environmental factors less influence in its etiology. Literature research found no mention of manubrium mesosternal joint fusion, either in biological distance studies or as a stress marker. Sternal aperture was successfully used in biological distance studies (Burrell 2018, McCarthy 2011, Ricaut *et al.* 2010).

Sternal caudal clefting is correlated with femur length, only for males, but the direction of this correlation is opposite to what expected. Males with sternal caudal clefting, tend to have longer femurs. Consequently, nothing indicates sternal caudal clefting might be used as a stress marker, and again, this might indicate this MCD is under strong genetic control. Notochord defect is correlated with tibia length, only

TABLE 4: Qui-square/Fisher's results test from MCD and cribra orbitalia and point-biserial correlation between MCD and femur, tibia and VNC size.

FEMALES									
	CO	**Femur**	**Tibia**	**AP Cerv.**	**AP Thor.**	**AP Lumb.**	**TR Cerv.**	**TR Thor.**	**TR Lumb**
Manubrium mesosternal joint fusion	p=0.384	r=-0.047; p=0.762	r=0.172; p=0.289	r=0.051; p=0.773	r=-0.011; p=0.957	r=0.083; p=0.616	r=0.270; p=0.117	r=0.171; p=0.385	r=0.055; p=0.749
Sternum hyperplasia	p=0.377	r=-0.159; p=0.261	r=-0.065; p=0.658	r=-0.138; p=0.384	r=-0.270; p=0.134	r=-0.136; p=0.372	r=-0.127; p=0.425	r=-0.282; p=0.118	r=-0.042; p=0.785
Sternal aperture	p=0.604	r=-0.017; p=0.905	r=-0.061; p=0.676	r=-0.043; p=0.786	r=-0.016; p=0.929	r=-0.054; p=0.727	r=-0.153; p=0.334	r=-0.195; p=0.284	r=-0.091; p=0.553
Sternal caudal clefting	p=0.237	r=-0.167; p=0.241	r=-0.230; p=0.116	r=-0.117; p=0.465	r=-0.079; p=0.673	r=0.273; p=0.137	r=0.188; p=0.239	r=0.273; p=0.137	r=-0.020; p=0.896
Notochord defects	p=0.495	r=0.219; p=0.082	**r=0.318; p=0.013**	r=-0.114; p=0.431	r=0.221; p=0.183	r=-0.063; p=0.645	r=0.183; p=0.204	r=0.267; p=0.105	r=-0.074; p=0.586
Hypoglossal canal	p=0.066	r=-0.086; p=0.501	r=-0.100; p=0.449	r=-0.099; p=0.496	r=-0.107; p=0.521	r=-0.208; p=0.124	r=-0.078; p=0.592	**r=-0.362; p=0.025**	r=-0.088; p=0.519
MALES									
	CO	**Femur**	**Tibia**	**AP Cerv.**	**AP Thor.**	**AP Lumb.**	**TR Cerv.**	**TR Thor.**	**TR Lumb**
Manubrium mesosternal joint fusion	p=0.669	r=-0.112; p=0.534	r=-0.017; p=0.926	r=0.267; p=0.218	r=-0.035; p=0.872	r=-0.031; p=0.865	r=-0.059; p=0.789	r=0.186; p=0.397	r=0.118; p=0.520
Sternum hyperplasia	p=0.711	r=-0.096; p=0.546	r=-0.064; p=0.689	r=-0.160; p=0.424	**r=-0.360; p=0.040**	r=-0.212; p=0.199	r=-0.354; p=0.070	**r=0.347; p=0.048**	r=-0.131; p=0.427
Sternal aperture	p=0.365	r=-0.016; p=0.920	r=0.054; p=0.736	r=-0.220; p=0.271	r=0.265; p=0.136	r=0.026; p=0.874	r=-0.381; p=0.050	r=0.028; p=0.879	r=0.155; p=0.348
Sternal caudal clefting	p=0.545	**r=384; p=0.012**	r=0.296; p=0.057	r=0.029; p=0.885	r=-0.036; p=841	r=0.015; p=0.929	r=103; p=0.610	r=-0.142; p=0.431	r=-0.065; p=0.695
Notochord defects	p=0.136	r=0.112; p=0.424	r=0.123; p=0.385	r=-0.187; p=0.297	r=-0.111; p=0.493	r=0.078; p=0.592	r=-0.003; p=0.985	r=-0.029; p=0.860	r=0.087; p=0.547
Hypoglossal canal	p=0.357	r=-0.167; p=0.232	r=-0.082; p=0.565	r=0.050; p=0.784	r=0.037; p=0.821	r=-0.072; p=0.618	r=298; p=0.092	r=0.072; p=0.659	r=0.158; p=0.274

in females, but again, females with notochord defects have longer tibias. Likewise, notochord defects do not prove to be useful stress markers. Sternum hyperplasia is correlated with AP and TR VNC diameters of thoracic vertebrae, in males. But, each of these correlations have an opposite direction. Males with sternum hyperplasia have narrower AP diameters and wider TR diameters. Considering AP diameters complete their growth, at approximately 4 years old, and TR diameters continues to grow up to 15–17 years old, possibly a narrower AP diameter is associated to a harsh environment early in life originating the presence of sternum hyperplasia; and a wider TR diameter might be linked to an improved environment during late childhood and adolescence, allowing for growth recovery. Therefore, use of sternum hyperplasia as stress marker might be defensible, although more studies are necessary to corroborate, or not, this correlation.

Double hypoglossal canal is correlated with TR diameters in thoracic vertebrae, in female. Females with double hypoglossal canal tend to have narrower TR diameters. From the six MCD analyzed, double hypoglossal canal is the most promising one to use as stress marker. Sex differences might be explained considering growing males were culturally more protected from harsh conditions than females (e.g. van Balen and Inhorn, 2003), although the privilege do not show in every MCD with significant results. Sternum hypoplasia is correlated with narrower diameters, only in males. Males are biologically less buffered than females to environmental stress during growth and development (Chen *et al.* 1981), but they are often culturally/behaviorally favored, which is a common phenomenon in patriarchal societies, where there is a preference in parental care towards male offspring, resulting in better feeding practices and use of medical care (Chen *et al.* 1981, Stinson 1985, van Balen, Inhorn 2003). Recent studies do not suggest a gender preference for boys in Portugal (Hank, Kohler 2000), nonetheless Cardoso (2005) did find a higher growth deficit in the non-adult female sub-sample from the Lisbon Collection. This correlation can also be a sign of accumulation, with individuals that went through stressful during embryogenesis, continuing to be affected with environmental insults during growth, which have affected TR diameters of thoracic vertebrae in females. If both sternum hyperplasia and double hypoglossal canal have a strong environmental etiology, the fact the first affects males and the second affects females, might be explained by other variables influencing expression of other stress markers under analysis, such as familial socioeconomic status that can have a strong effect on growth.

In a previous study (Amoroso 2020), a comparison of prevalence rate of several MCD with other studies, found that the Lisbon sample had statistically significant lower crude prevalence in most dyads compared. For instance, manubrium mesosternal joint fusion had lower prevalence than two contemporary samples of Istambul (Turkay *et al.* 2017, Yekeler *et al.* 2006); sternal aperture had lower prevalence than contemporary samples from Brazil, India, Kenya and Black Americans (Babinski *et al.* 2012, 2015, Boruah *et al.* 2016, El-Busaid *et al.* 2012, Donlon 2000); sternal caudal clefting also has lower prevalence rates than a Chinese contemporary sample (Donlon 2000). Only in a study from Dallas, USA, from AD 1350–1450, prevalence rates of manubrium mesosternal joint fusion where higher in the Lisbon sample (McCarthy 2011).

Considering the Lisbon sample is from early twentieth century, when Portugal was experiencing strong political and economic difficulties, results are unexpected, unless life conditions in comparative samples were not expressively different. A methodological reason might be an alternative justification for these differences. Many of the studies mentioned above used thorax MDCT evaluations of patients who took the exam for several medical reasons (e.g. investigate primary or metastatic tumors, vascular and airway pathologies) (Yekelar *et al.* 2006, Babinsky *et al.* 2015, Turkay *et al.* 2017). On one hand these were biased samples, on the other, the two methodologies of bone analysis might not be comparable.

The ANOVA failed to prove any age-at-death differences between groups, according to the timing of formation of their stress markers. The main reasons for these unexpected results might be the uneven composition of the groups. In the group of individuals with stunt VNC, femur or tibia size was comprised by only four individuals. Other reasons might hinder the effect of stress markers in age-at-death, namely the ability to catch-up-growth and the presence of confounding variables not tested, such as socioeconomic status.

Although research regarding the possible relation between MCD and other stress markers is not new, more studies to consolidate the idea that MCD are useful to test the accumulation disadvantages throughout the life course hypothesis are still lacking. Results obtained might not seem very promising, but

MCD were not used discretely in biological distance studies, thus it is impossible to assess the independent contribution of each one. Each MCD must be analyzed alone, because it is the only way to identify a new, formed in uterus, stress marker.

CONCLUSION

Six Minor Congenital Defects were tested as possible stress markers. Sternum hyperplasia and double hypoglossal canal might have potential to capture environmental stress in utero, because they are correlated with other stress markers. Manubrium mesosternal joint fusion, sternal aperture, notochord defects and sternal caudal clefting do not gather any evidences of a strong environmental impact on its etiology, and therefore this study suggests they are not useful to document stress early in life. Considering this is the first study to identify the potential of sternum hyperplasia and double hypoglossal canal as stress markers, further investigation should be done in other samples, from different sites and chronologies.

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