SEX DIFFERENCES IN ORAL HEALTH IN THE EARLY MEDIEVAL NON-ADULT POPULATION FROM ZNOJMO-HRADIŠTĚ, CZECH REPUBLIC

Pohlavní rozdíly ve stavu chrupu u časně středověké nedospělé populace ze Znojma Hradiště, Česká republika

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Abstract

The present study aimed to compare dental caries and LHPC (localised hypoplasia of primary canines) on deciduous and permanent teeth in male and female non-adult individuals of the Slavic population from the 9th to the first half of the 10th century CE in the South Moravian burial ground of Znojmo-Hradiště, Czech Republic. The sex of the 37 non-adult individuals was molecular-biological determined. This analysis allowed to compare the incidence of the dental caries and LHPC between both boys and girls. The macroscopy method was used to detect dental caries and only cavitations were count as tooth decay. LHPC was searched for via oblique light, zoom and stereomicroscope respectively. The differences between the males' and females' oral health were statistically tested. The distribution of dental caries within the male and female groups shows clearly worse oral health among girls. The statistically significant differences were found between numbers of males' and females' carious teeth among both, deciduous and permanent teeth. The incidence of LHPC in males' primary canines is statistically significantly higher: 41.18% of males' canines were affected by LHPC, 20.83% of females' canines were affected by LHPC. Some studies of the recent populations have not evinced the difference between boys and girls in the incidence of the dental caries in non-adults. In our study, the difference could be influenced by a low number of the individuals or some of the discussed genetic, behavioural or hormonal reason. We reported only preliminary results and this study will continue.

Key words: *dental caries, localised hypoplasia of primary canines, molecular-biological determination of sex, Slavic population*

Introduction

The site Znojmo-Hradiště Svatého Hypolita (Stronghold of St. Hyppolytus, 48°51'37.0"N 16°01'37.0"E) is located on the south-west margin of the town of Znojmo. The geographic location of this heavily fortified Great Moravian stronghold is on the top of a strategically important rocky hill at an elevation of 332 m. It is protected by the deep valleys of the Dyje River and the streams Gránický and Pivovarský from three sides. The climate is warm in the summer with a mean air temperature of 22–25 °C, and a mean annual precipitation of 546 mm. Winters are the dryer seasons with mild mean air temperatures of 2–4 °C. The soil is fertile alkaline black earth (Wikipedie, 2017). The site has been continuously inhabited since the Late Stone Age. The whole fortified area stretched over 20 hectares in the 9th century and it is therefore considered to be one of the largest Moravian strongholds. In its time, which can be considered the most glorious period of Moravian history, this place ranked among only a few centres of political power, economy, religion and culture, that formed the backbone of the Great Moravian Empire, the oldest state system of the Western Slavs. Proof of its size, maturity and vital importance for other Slavic regions has been shown in archaeological findings. The Department of History, Faculty of Education at Masaryk University in Brno has been carrying out systematic research at the Great Moravian hilltop of the St. Hippolytus stronghold for more than two decades. These studies have already brought many interesting and important findings that have enriched our understanding of the earliest Czech history. The biggest discoveries ever of the last few years certainly include that of a rich central burial ground/necropolis from the 9th and first half of the 10th centuries. This was, based on the evidence already obtained, the eternal resting place of more than 1,500 Moravian individuals. After 22 years of intensive research, we managed to begin examination of the western foreland of the Hradiště Stronghold and gradually explore it. Seven years of research have so far brought a thorough investigation, versatile documentation and a collection of nearly 600 rich skeleton graves, which covered about one third of the assumed/anticipated area of the necropolis. This would mean that the necropolis – with its unique findings of material culture showing a close relationship to the wider Central European region and the middle Danubian area and the eastern Cisalpine (Předalpí) – ranks among the largest and most important necropolises dating from the 9th and the first half of the 10th centuries in the Czech Republic.

The rectangular graves of examined individuals were located at a shallow depth on the bottom of the topsoil without digging out to the loessial bedrock. In spite of the high density of graves, the younger graves almost perfectly avoided damage of the older ones. A number of graves had been marked with some kind of wooden indicator; probably there were wooden constructions above several graves. Charitable gifts were frequent in the graves of these individuals, with the exception of grave number 485 (a 2-year-old boy with LHPC and dental caries) where only a small stone ball and ash were found, grave number 442 (7-year-old girl with dental caries) and grave number 545 (14-year-old girl in the right side position with crossed arms and bent legs). Among the simpler gifts there were numerous knives, eggs and bones from domestic fowl and possibly smaller pottery containers. The richer grave inventory then included other examples like small bells, buckles, glass bottoms and beads, rings and especially various types of bronze and silver earrings, which were found in the graves of both girls and boys surprisingly often (e.g. Gr. No. 499, 502, 538b, 556). Every individual was committed to the grave on his/her back with exception of two individuals from grave number 545 (on the right side) and grave number 556 (a 9-year-old boy in prone position), some of them in wooden coffins.

The results of the anthropological research of the juvenile part of population from this Early Medieval site show the highest mortality in the age category Infans I (0.5–6 years) (Drozdová, 2011). Sex was determined by methods of molecular biology in the 37 juvenile individuals coming from the Znojmo-Hradiště site. The aim of the molecular biological determinations of the sexual structure of boys and girls was to draw attention to the fact that the possibility of determining the sex of children's skeletal remains will bring new possibilities to the study of these remains (Drozdová, Brzobohatá, & Klíma, 2017). In this study we focused on the evidence of possible sex differences in the incidence of dental caries and LHPC (localised hypoplasia of primary canines) on non-adults. The existence of sex differences in the oral health of adult individuals is well documented in a large number of studies, e.g. Lukacs (2008), in North America (Larsen, 1998; Walker & Erlandson 1986), in South Asia (Lukacs, 1996) and a meta-analysis of caries prevalence in prehistory (Lukacs & Thompson, 2007). A significant decline in oral health is mainly a consequence of women's pregnancy resulting from a complex of genetic, behavioural and hormonal factors (Acharya, Bhat, & Acharya, 2009; Covington, 1996). X-linked genetic variation influencing the oral environment, the genomic diversity of oral bacteria and a microstructure of enamel may partly explain the origination of sex differences in oral health (Lukacs, 2011; Miller, 2009). The genetic study from the Philippines found a caries-suggestive loci for genes influencing saliva flow and diet preferences (Vieira, Marazita, & Goldstein-McHenry, 2008). This study, focused on a genetic variation of candidate genes involved in an amelogenesis, hypothesizes that these genes contribute to micro-structural alteration in enamel that may result in higher mineral loss under acidic conditions and may facilitate bacterial attachment to biofilms (Deeley et al., 2008; Patir et al., 2008). The behavioural factors are gender-based dietary preferences and a culture-based division of labour. The magnitude of the disparity in oral health according to sex increases during ontogeny and there is an association of age and parity. Women's oral health declines more rapidly than men's with the onset of agriculture and the associated rise in fertility (Lukacs, 2011; Wesolovski, 2006).

The lower income „prestigious" (eg. meal) is one of the reason for increased dental caries among women from agricultural societies in comparison with men, which is usually replaced by the increased income in carbohydrates. In view of this fact we wanted to determine whether discrepancies in the food are pronounced in the dental health of children and adolescents. Genetic effects (e.g. taste preference) could become evident already among children or adolescents, these are independent on reproduction and parity, as we mentioned above.

Localised enamel hypoplasia of the human primary canine (LHPC) is an area of defective enamel formation on the labial surface of the crowns of primary canines that was first reported by Jørgensen (1956). The size of such a defective area is usually 1–2 mm with a flat base. Most cases of LHPC are solitary, but multiple lesions on one tooth have been observed in both prehistoric and modern populations. Genetic and environmental factors are considered to be important in the aetiology of the lesion (Jørgensen, 1956; Taji, Hughes, Rogers, & Townsend, 2000; Mukhopadhyay, Roy, Mandal, Ghosh, & Chakraborty, 2014) as well as minor trauma to the developing canine tooth bud (Skinner, 1986) and harsher environmental conditions shortly after birth connected with insufficient nutrition and poor state of health of the mother (Skinner & Hung, 1989; Taji et al., 2000).

We have drawn on the 37 non-adult individuals from Znojmo-Hradiště with molecular-biological determined sexual structure to search for sex differences in oral health. Sex differences in non-adults have been documented very rarely in the literature and therefore a comparison with other sets is difficult.

Aim

The present study aimed to compare the incidence of dental caries and LHPC (localised hypoplasia of primary canines) on deciduous and permanent teeth in male and female non-adult individuals of the Slavic population from the 9th to the first half of the 10th century CE in the South Moravian burial ground of Znojmo-Hradiště, Czech Republic. The sex of the 37 non-adult individuals was molecular-biological determined.

Material and Methodology

We examined primary and permanent teeth of the 37 non-adults with molecular-biological determined sex from the burial site Znojmo-Hradiště excavated up to 2007–2009 (Drozdová et al., 2017). The examined sample consists of 14 boys and 23 girls. The boys died prevalently in the age categories of Infans I and Juvenis, while the girls died most often in the age category Infans II (7–13 years).

The dental examination was carried out closely by one examiner to eliminate inter-observer error, using oblique light, zoom and a stereo-microscope. The teeth were rotated at various angles to highlight areas of deficient or defective enamel. The lack of continuity of the surface enamel was recorded as hypoplasia, according to the DDE index (the standardised code from the epidemiological index of developmental defects: D-hypoplasia – pits and G-hypoplasia – missing enamel) (FDI Commission on Oral Health, Research and Epidemiology, 1992, 423). We noted the location on the type of canine, i.e. maxillary or mandibular and left or right site. We then pinpointed the shape of the hypoplastic defect as round, oval, triangular or irregular following Taji et al. (2000) and localization on the labial surface of canine. This surface we have divided into thirds vertically (cervical, middle and incisal in the inciso-cervical plane) and horizontally (mesial, middle and distal in the mesiodistal plane) following Wheeler (1969).

The instances of tooth decay were located by visual macroscopic method; in the case of confusion a dental probe was used. We recorded for each individual the teeth presented in the jaws, the enclosed teeth fallen out of the alveoli post mortem, the teeth with tooth decay, the number of cavities in the one tooth, and location of caries on the root or on the crown. The tooth surfaces affected by caries and the approximate size of the cavity were documented.

Statistical analysis was carried out using a Wald-Wolfowitz test with a level of significance of 0.05. The distribution of data was tested by the K-S & Lilliefors normality test and there was not a normal distribution of data. The Wald-Wolfowitz test is a non-parametric analogy of the T-test for two independent samples. It tests a distribution of two samples as well as proportions. This test does not have any additional requirements such as the Mann-Whitney test which requires the same proportions of data in groups and this was not the case in our analysis. The program STATISTICA CZ 12 (StatSoft, Inc. 1984–2013) was used for statistical analysis.

Results

In total we analysed 37 non-adult individuals, but only 31 had some observable deciduous or permanent teeth. The observed minimum of teeth was four per individual. Three individuals were newborn with no observable dentition and two individuals did not have any preserved teeth. The age structure of the analysed file is shown in Table 1. The sex distribution is 12 boys and 19 girls with preserved dentition, 2 male newborns and 1 female newborn without erupted dentition and 3 girls (aged 1.5, 5–6 and 7 respectively) with no preserved dentition. In total we found 625 preserved alveoli of both primary ($n = 204$) and permanent teeth ($n = 421$) in this analysed file. Table 1 shows the distribution of preserved deciduous and permanent teeth in the particular age categories Infans I, II and Juvenis in connection with the molecular biological determined sex of the observed individuals. With regard to primary teeth,

***Table 1.** The total counts of individuals in the age categories, numbers of deciduous and permanent alveoli and numbers of primary and secondary teeth*

Classification (Age)	Individuals			Deciduous alveoli						Permanent alveoli						Deciduous teeth						Permanent teeth					
				Maxilla			Mandible			Maxilla			Mandible			Maxilla			Mandible			Maxilla			Mandible		
	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.	M	F	Tot.
Circumnatale (0–0.5)	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infans I (0.5–6.9)	5	7	12	27	17	44	30	32	62	2	2	4	2	0	2	35	19	54	44	31	75	2	0	2	2	0	2
Infans II (7.0–13.9)	3	9	12	15	35	50	4	44	48	14	43	57	20	42	62	5	23	28	4	30	34	25	60	85	15	56	71
Juvenis (14.0–20.0)	4	6	10	0	0	0	0	0	0	52	94	146	54	96	150	0	0	0	0	0	0	48	73	121	56	74	130
Total	14	23	37	42	52	94	34	76	110	68	139	207	76	138	214	40	42	82	48	61	109	75	133	208	73	130	203

Note. F = female; M = male; Tot. = total.

the most numerously represented category is Infans I, the group of individuals from half a year to seven years old. There were 54 analysed primary teeth of the upper jaw and 75 teeth of the lower jaw of both boys (n = 79) and girls (n = 50) in this group of individuals. The most numerous counts of permanent teeth were found in the category Juvenis with individuals from 14 to 19 years old. We analysed 121 permanent teeth of the upper jaw and 130 teeth of the lower jaw of both males (n = 104) and females (n = 147) in this group of individuals. In total we analysed 602 teeth both deciduous and permanent.

The distribution of dental caries within the male and female groups (Table 2) shows clearly worse oral health among girls. We found 15 dental caries in the girls' deciduous teeth with a numerical superiority in the upper jaw teeth and 9 dental caries in the girls' permanent teeth with a numerical superiority in, on the contrary, the lower jaw teeth. Only 2 male deciduous maxillary teeth and1 male mandible permanent tooth evinced dental caries. The differences between male and female carious teeth are statistically significant in both deciduous and permanent teeth. The distributions were tested by the K-S & Lilliefors normality test, however there were not normal distribution in these files. Therefore the non-parametric Wald-Wolfowitz test was used to statistical test:

- the incidence of dental caries in the female upper primary teeth is statistically significantly higher than caries incidence in the male upper primary teeth (p = 0.014210),
- the incidence of dental caries in the female lower primary teeth is statistically significantly higher than the caries incidence in the male lower primary teeth (p = 0.000024),
- the incidence of dental caries in the female upper permanent teeth is statistically significantly higher than the caries incidence in the male upper permanent teeth (p = 0.000220),
- the incidence of dental caries in the female lower permanent teeth is statistically significantly higher than the caries incidence in the male lower permanent teeth too (p = 0.008553) (Figure 1).

LHPC was displayed in the primary canines of 3 (25.00%) boys and 3 (15.79%) girls (Table 3). The seventeen males' primary canines both upper (n = 8) and lower (n = 9) were preserved in the analysed collection. The seven (41.18%) of these canines were affected by LHPC, 3 (37.50%) upper canines and 4 (44.44%) lower canines. The twenty four females' primary canines both upper (n = 9) and lower (n = 15) were preserved, 2 (22.22%) upper canines and 3 (20.00%) lower canines displayed LHPC, 5 females' canines in total (20.83%). The differences between the male and female primary canines with localised enamel hypoplasia are statistically significant (upper primary canines – not normal distribution by the K-S & Lilliefors normality test, non-parametric Wald-Wolfowitz test, p = 0.000839; lower primary canines – not normal distribution by the K-S & Lilliefors normality test, non-parametric Wald-Wolfowitz test, p = 0.008553) (Figures 2, 3).

Only one boy, two years old (Gr. No. 485), carried dental caries on his upper first incisors. This was contact caries between the distal surface of *i1dexter* and mesial surface of *i2 dexter*, the cavitations are about 2 mm in size. Both lower primary canines and one preserved upper canine of the boy carried LHPC. This individual was buried probably without any grave gifts, only one small stone ball and ash was found in the grave inventory. An eighteen-year-old male was the second male individual displaying tooth decay (Gr. No. 429). This was a fissural caries located on the occlusal surface of the second right lower molar. The other permanent teeth of this male had been affected by linear hypoplasia. He had been laid in supine position in the grave that was marked by some wooden indicator behind his head. Only a knife was found in the grave's inventory.

Table 2. *Distribution of dental caries within male and female groups*

Dentition	Sex	Maxilla caries (*N*)	Mandibula caries (*N*)	Total caries (*N*)	Total of teeth (*N*)	I-CE	Individuals with dentition (*N*)	Individuals with caries (*N*)	F-CE (%)
Deciduous	male	2	0	2	88	2.0	7	1	14.3
	female	10	5	15	103	15.0	11	5	45.5
Permanent	male	0	1	1	148	0.7	8	1	12.5
	female	2	7	9	263	3.0	14	3	21.4
Total		14	13	27	602	4.5	31	9	29.0

Note. F-CE = frequency of caries, I-CE = index of caries, N = number.

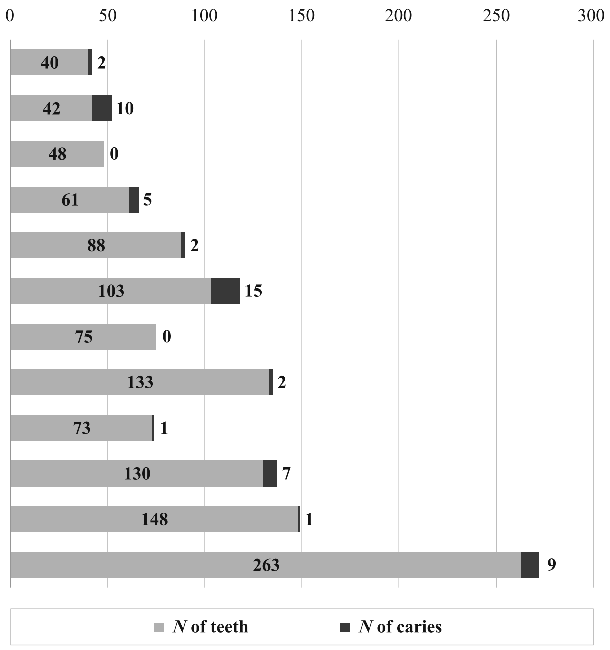

Figure 1. Graph of numbers of male (M) and female (F) deciduous (dec) and permanent (per) teeth in upper (*max*) and lower (mand) jaws in the analysed file with indication of number (*N*) of dental caries.

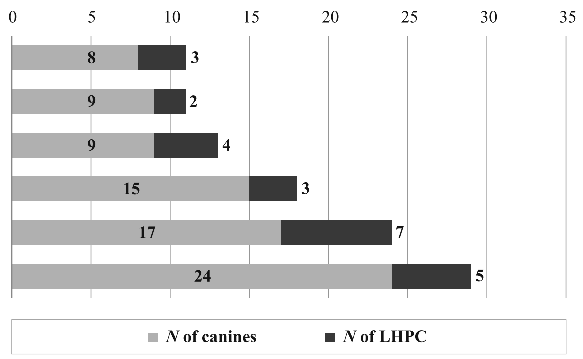

Figure 2. Graph of the numbers of male (M) and female (F) deciduous (dec) canines in upper (*max*) and lower (mand) jaws in the analysed file with indication of number (*N*) of LHPC (localised hypoplasia of primary canines).

In the group of eleven girls with some preserved deciduous teeth, we found 5 girls with at least one dental caries. Among these were four individuals with drastically inferior oral health. Their deciduous teeth carried several caries and the primary canines of three of them display LHPC (Table 3) besides this the enamel of the other primary teeth have been affected by hypoplastic pits.

The youngest was a 2-year-old girl (Gr. No. 522) who was buried on her back with pottery containers and seeds and shells within. Some animal bones were found in this grave which was also marked by some wooden indicator behind her head. There were 13 deciduous teeth preserved within her jaws which displayed several dental caries. The first is located on the upper left first molar (*m1 max sinister*) in the form of a 2-millimetre-large cavitation like a mustard seed. The right upper incisors of the individual are dark-coloured with partial *post mortem* damage, but it cannot be excluded that the incisors have been affected by tooth decay under the gingival line, being suggestive of today's early childhood caries (ECC).

The 7-year-old girl (Gr. No. 442) had been placed on her back with a stone above her spine and pelvis. Her grave was also marked by some wooden indicator. No charitable gifts were found in this grave. The fifteen deciduous teeth were localised in her jaws. The left upper molars were affected by contact decay on the distal and mesial surface. Her upper incisors are worn strongly, but nevertheless we assumed according to the damaged enamel and black colour of the dentine, they would have been affected by tooth decay under the gingival line (Figure 4). The enamel of her other deciduous teeth shows the presence of hypoplastic pits and the enamel of her permanent teeth (all the first molars) carries linear hypoplasia.

The molars of all of the other three girls (8 years old – Gr. No. 505; 9 years old – Gr. No. 519; 10–11 years old – Gr. No. 508) were affected by contact or fissural tooth decay and the primary canines displayed LHPC. We have found seven molars in the jaws of the first girl (Figure 5). Three preserved primary canines show LHPC and the enamel of her twelve erupted permanent teeth is affected by linear hypoplasia. She was buried on her back with an iron knife and bronze earrings and nearby grape seeds had been found.

The second girl had three upper and four lower molars preserved. All the upper molars had been affected by caries on the contact surfaces, both mesial and distal. The lower molars were healthy, but all the primary teeth displayed small pits in the enamel and all three preserved primary canines show LHPC (Figure 6). All of the ten erupted permanent teeth of this girl show linear hypoplasia. This girl was buried in a wooden coffin in supine position with an iron knife, pottery container and some kind of bird.

Only the lower molars have been preserved of the last girl. All of them are affected by serious tooth decay on the contact and occlusal surfaces, and some of them had afflicted the pulp cavity. The upper right primary canine shows LHPC and all the anterior permanent teeth show linear hypoplasia. The upper left permanent first molar had been affected by tooth decay

Figure 3. Grave No. 538A (the 1.5-year-old boy), the lower primary canines are affected by LHPC while no caries was found on the teeth of this individual.

Figure 4. Grave No. 442 (7-year-old girl), upper incisors are strongly worn.

Figure 5. Grave No. 505 (8-year-old girl), six of her molars are afflicted by extensive caries, some of which had reached the dental pulp and had destroyed a large part of tooth.

in a fissure on the occlusal surface (Figure 7). This girl was laid on her back with bronze earrings and a pottery container in the grave.

The first and second molars and one premolar as well of the other two girls, whose deciduous dentition has not been preserved, have been affected by caries. The first one (Gr. No. 446, the girl aged 13–14) has preserved eight permanent first and second molars and eight premolars. The second lower left premolar had been affected by contact decay on the distal

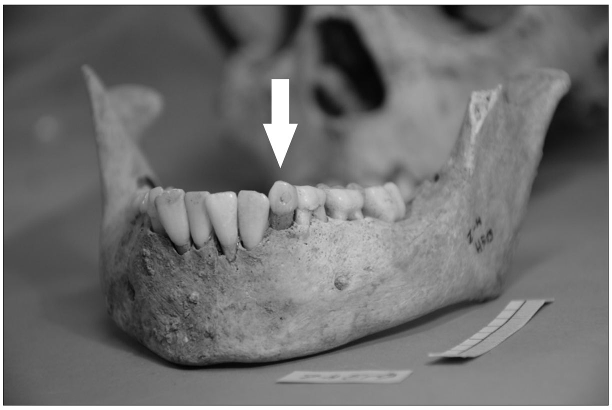

Figure 6. Grave No. 519 (the 9-year-old girl), LHPC of the lower primary canine.

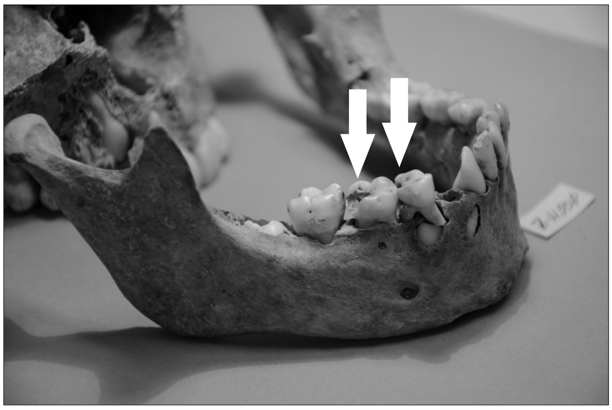

Figure 7. Grave No. 508 (the girl aged 10–11), lower left molars affected by contact tooth decay on distal, mesial, and occlusal surfaces.

surface and first upper right molar, both first lower molars and the right lower second molar show both contact and fissural caries on the mesial and distal surfaces and the occlusal surfaces as well. This girl was laid on her back with bronze rings and earrings, two small knives, a chicken egg and shells of small molluscs in the grave. The second girl (Gr. No. 467), aged 12–15, has no caries on the upper teeth, but the lower molars (*M1* and *M2 dx*, *M1 sin*) have been affected by dental caries in their *foramen caecum* on the buccal surfaces and there the left lower molar has been affected by fissural decay on its occlusal surface. The permanent teeth show linear hypoplasia. This girl was laid on her back also with a bronze earring and ring, glass beads and a chicken egg in the grave, which was marked by some wooden construction above it.

Discussion

It has been widely documented that dental caries rates increased with the adoption and intensification of agriculture (Ettinger, 1999; Larsen, 1983; Larsen, Shavit, & Griffin, 1991; Lukacs, 1992). Higher caries prevalence among females is explained by several factors with regard to the deciduous dentition. Usually the earlier eruption of teeth in girls poses the first impact, as it brings longer exposure to the cariogenic oral environment (Lukacs & Largaespada, 2006). The following factor could impact the oral health of primary dentition among societies, in which girls used to be close to their mothers helping with everyday domestic chores from an early age, most importantly cooking in this context. The traditional anthropological explanation is the proximity of women to food sources and snacking during cooking or preparation of meals (Lukacs & Largaespada, 2006). Boys of the same age would rather be close to their fathers in helping with male labour. The main impact is attributed to the sexual division of labour and women's domestic role in food production in this behavioural explanation (Kelley, Levesque, & Weidl, 1991). Besides the culture-based division of labour, gender-based dietary preferences have been included among behavioural factors (Lukacs, 2011). A combined high-carbohydrate and low-protein diet in females may have predisposed their teeth to more decay (Larsen, 1995).

Pregnancy is the third frequent explanation of sex differences in oral health, but it is unreasonable among children. Nevertheless fluctuating hormone levels during individual life histories could be taken into consideration. The biochemical hormonal fluctuations during events such as puberty and menstruation, making the oral environment significantly more cariogenic for women just as pregnancy does, could serve as an explanation of the sex differences on permanent dentition in older girls compared with boys. Clinical research, beginning in the 1990s, revealed that the biochemical composition of saliva and rate of flow vary significantly by sex and play a prominent role in causing sex differences in oral health generally, and differences in dental caries rates in particular (Lukacs & Largaespada, 2006). Hormonal fluctuation during a person's life history may have influenced the biochemistry of saliva, chemical composition and flow rate of saliva and may have caused changes to dental caries rates, creating a lower incidence among males and a higher incidence among females. Girls showed a higher prevalence of caries than boys at each age in the study of Mansbridge (1959) on Edinburgh schoolchildren aged 7–12. In subsequent studies, these differences have not been suppressed even with the frequent use of fluoride toothpaste by girls (Lukacs & Largaespada, 2006); the gender differences in fluoride exposure was too small to matter, or the role of fluoride toothpastes as a causal factor in caries decline was overrated (Haugejorden, 1996).

Saliva has seven primary functions:

1) *protection* – a mechanical washing action that flushes away cellular debris and sugars from the oral cavity, thus reducing their availability to acidogenic bacteria that cause demineralization of the enamel. Calcium-binding proteins in saliva help from the salivary pellicle, a protective membrane-like coating that protects the outer enamel surface;
2) *buffering* – maintenance of an optimal environmental of pH denying bacteria, neutralization of acids produced by bacterial microorganisms in plaque, prevention of demineralization and cavitation of enamel;
3) *antimicrobial action* – proteins in saliva have a dramatic influence on the ecology of the oral cavity (lysozyme, lactoferrin, etc.);
4) *tooth integrity* – calcium and phosphate ions in saliva assist in post-eruptive maturation of the enamel, increases the hardness of enamel and decreases the permeability of the outer enamel surface;
5) *digestion* – saliva facilitates the sense of taste, dissociation of starch and formation of food bolus;
6) *taste* – saliva dissolves substances so that they can be tasted and carries substances to the taste buds, namely gustin, a protein partly responsible for the growth and maturation of taste buds; and finally
7) *tissue repair* – shorter bleeding, faster healing, as yet unidentified clotting factors (Lukacs & Largaespada, 2006).

Males and females both have different levels of the two key groups of gonadal steroid hormones: estrogens and androgens. Research performed on laboratory animals reveals that caries rates increase in proportion with increasing estrogen levels, whereas increasing androgen levels have no effect (Delman, 1955; Laine et al., 1988; Liu and Lin, 1973; Legler & Menaker, 1980). Several animal studies have found a connection between increased thyroid levels in the blood and a decrease in carries rate (Muhler & Shafer, 1955; Ryan & Kirkwood, 1955). Fluctuations in the level of estrogens influence thyroid activity and lead to a reduction in the saliva flow rate, and an increase in the caries rate (Delman, 1955; Muhler & Shafer, 1955). Estrogen levels are significantly higher during puberty, pregnancy, and menstruation among women. While males have small amounts of estrogen, research confirms that estrogen levels are higher in females throughout the life cycle (Angsusingha, Kenny, Nankin, & Taylor, 1974; Niswender, Abulfatah, & Nett, 1976; Worthman, 1995).

Sex differences in the composition and flow rate of human saliva have been proven by studies. The composition of saliva (its quality, i.e. its level of minerals, amount of microbial constituents, buffer capacity) and the quantity of saliva (average of flow rate) do not have an equal influence on oral health. If saliva quality is high but saliva flow rate is low, then oral health can still be negatively impacted. Saliva quality and quantity differ on an individual basis, and may be influenced by factors such as malnutrition (Lingstörm & Moynihan, 2003). However, the quality and quantity of saliva vary even more dramatically between males and females, and in response to certain life-history events. One might attribute this to the fact that females' salivary glands are smaller, but this could not cause such a difference in itself. Probably estrogen plays a role. One possible mechanism underlying these relationships is suggested by the presence of estrogen-receptor mRNA and immunoreactive estrogen-receptor protein in the saliva glands and oral mucosa, implicating the influence of estrogens on oral health (Leimola-Vitranen, Salo, Toikkanen, Pulkkinen, & Syrjänen, 2000).

A genome-wide scan finds suggestive caries loci. Evidence from twin studies suggests a genetic component to caries (Boraas, Messer, & Till, 1988; Conry, Messer, Boraas, Aeppli, & Bouchard, 1993). The genetic contribution to caries has been estimated to be 40% (Conry et al., 1993) or 45–64% (Bretz et al., 2005). Suggestive loci have been determined for low caries susceptibility (5q13.3; 14q11.2; Xq27.1) and high caries susceptibility (13q31.1 and 14q24.3). Genes that may be related to saliva flow and diet preferences have been proposed as possible candidates. A protective locus for caries in the X chromosome may explain the gender differences seen in caries frequency (Vieira et al., 2008). Tuftelin genotypes appeared to interact with levels of Streptococcus mutans infection in children with ECC compared with caries-free children (Slayton, Cooper, & Marazita, 2005). Nariyama, Shimizu, Uematsu and Maeda (2004) reported that the only possible candidate gene on the mouse genome-wide scan that maps to human 14q11.2 is NFATC4 (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4). According to the authors this gene is involved in intra- and extracellular transport of calcium and potassium ions relating to the flow rate of saliva. The marker in 14q11.2 (D14S742) is very close to OR4E2 (olfactory receptor, family 4, subfamily E, member 2) which interacts with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell. Taste and smell are subsumed under the term "flavour". Many flavours are recognized mainly through the sense of smell. Genetic variation in genes regulating olfactory and taste sensations may predispose someone to be more or less inclined to eat certain foods, and therefore to have a less or a more cariogenic diet. CARTPT (cocaine- and amphetamine-regulated transcript) is located in between the markers in 5q13.3 (D5S2500 and D5S424). This gene has a role in reward, feeding, and stress, and it has the functional properties of an endogenous psychostimulant (Kuhar, Adams, Dominguez, Jaworski, & Balkan, 2002). Decreased ingestion of sweet foods in behaviour would contribute to a lower caries experience. The human X chromosome carries a protective locus for caries. This finding could help to clarify the gender differences in caries prevalence. X-linked genetic variation could partly explain why men tend to have fewer caries than women. One of the regions with the most significant results for the "higher caries experience" scan was 13q31.1. The marker (D13S317) is near SPRY2 which has a bearing on physiological responses, including immune responses. SPRY2 is an antagonist of FGF signalling, one controller of the integrity of oral mucosa which has mitogenic effects in the salivary glands as well (Kagami et al., 2000). The second region with the most significant results was 14q24.3 which has a marker (D14S53) close to ESRRB (estrogen- related receptor beta). This gene encodes a protein with similarity to the estrogen receptor. Its function is unknown (Zhou et al., 2006). Estrogens reduce the secretion of growth hormone, which is closely related to the development and maintenance of the normal histologic structure of salivary glands. Their function can influence caries formation (Liu, 1967).

Arantes, Santos, Frazao and Coimbra (2009) gathered caries data in the Xavante of the village of Etenhiritipá, an indigenous group of central Brazil. They used four age groups (6–12, 13–19, 20–34, 35–60). In all age groups, mean DMFT (decayed, missing, and filled teeth) is higher in women than men, but significant gender differences only occur in the 13–19 age range in and the 20–34 age group. Gender differences in mean DMFT in Hungary among six age groups (≤19, 20–34, 35–44, 45–64, 65–74, ≥75) were highly significant in all age groups except the youngest (≤19 years) and oldest (≥75 years) (Lukacs, 2011; Madléna, Hermann, Jáhn, & Fejérdy, 2009). An extensive meta-analysis of over 50 clinical and epidemiological studies of caries experience in South Asia shows that in the younger age group mean DMFT (deciduous teeth) in males is greater than, or equal to, the female mean (Lukacs, 2011). The results of these studies did not evince great differences in dental caries prevalence between non-adult men and women.

In our study on the file from Znojmo-Hradiště differences exist between the incidence of caries in males' (I-CE = 2.0) and females' (I-CE = 15.0) deciduous teeth. The deciduous teeth are present in the age categories Infans I and II, which include individuals to the 14 years-old. The oldest girl bearing deciduous caries was 10–11 years-old. Thus, we can exclude the influence of pregnancy to the females' worse oral health. The discussed other reasons such as genetic influence or different quality and quantity of saliva could be the causation of this effect. The incidence of caries in males' (I-CE = 0.7) and females' (I-CE = 3.0) permanent teeth is less different as on deciduous teeth, however males still bear less caries than females. The oldest girls bearing caries on the permanent teeth were 14–15 years-old. It is debatable whether they could be pregnant in this low age. Unfortunately, on this burial site are not yet processed the analysis of the food resources of the population, nor the types of food that would confirm or refute the differences in the diets of males and females. We cannot, therefore, assess the difference in caries frequency in this context. Analysis of diet, e.g. using analyse of isotopes, are in the plan. Similarly, the comparison with other Slavic burial sites of the same period at the moment when will be molecular-biological determined sex of the larger number of individuals.

Conclusion

In our study on the file from Znojmo-Hradiště differences exist in oral health between male and female non-adults. The girls have shown a statistically significantly higher incidence of dental caries. The boys, on the other hand, have shown a statistically significantly higher incidence of LHPC. The reason could be the small number of individuals in the analysed file, only 31 individuals with some preserved teeth, the harsher environmental conditions of girls, other type of diet or behavioural differences in males and females respectively. We reported only preliminary results and this study will continue.

Souhrn

Cílem naší studie bylo porovnat orální zdraví nedospělých jedinců z pohřebiště Znojmo-Hradiště, které patřilo slovanské populaci 9. a první poloviny 10. století. Nachází se u významného velkomoravského opevněného hradiště na skalní ostrožně západně od města Znojma. U 37 nedospělých jedinců bylo molekulárně-geneticky stanoveno pohlaví, které morfoskopickými metodami nelze s jistotou určit. Tato analýza otevřela možnost porovnání výskytu zubního kazu a lokalizované hypoplazie primárních špičáků u chlapců a děvčat z tohoto pohřebiště. Z těchto 37 jedinců mělo pouze 31 zachovánu dentici. Srovnání ukázalo statisticky významně vyšší výskyt zubního kazu u děvčat a naopak statisticky významně vyšší výskyt lokalizované hypoplazie primárních špičáků u chlapců. Srovnání výsledků s jinými studiemi je obtížné, neboť přesné stanovení pohlaví u nedospělých jedinců je ojedinělé. Srovnání je možné s výzkumy recentní populace. Některé tyto studie nevykazují rozdíl ve výskytu zubního kazu u nedospělých jedinců. Rozdíl v naší studii může být způsoben analyzovaným malým souborem anebo je důvodem právě některá z diskutovaných skutečností genetického, behaviorálního nebo hormonálního charakteru. Zdá se, že velkou úlohu v protekci zubních kazů hraje i kvalita a kvantita slin, která je u mužského pohlaví vyšší.

Klíčová slova: *zubní kaz, lokalizovaná hypoplazie primárních špičáků, molekulárně-biologicky stanovené pohlaví, slovanská populace*

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