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Dental stigmata and enamel thickness in a probable case of congenital syphilis from XVI century Croatia

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ABSTRACT

Objective: To analyse the dental remains of an individual with signs of congenital syphilis by using macroscopic observation, CBCT and micro-CT images, and the analysis of the enamel thickness.
Design: Anthropological analysis of human skeletal remains from the 16th century archaeological site Park Grič in Zagreb, Croatia discovered a female, 17–20 years old at the time of death, with dental signs supportive of congenital syphilis: mulberry molars and canine defects, as well as non-specific hypoplastic changes on incisors. The focus of the analysis was on three aspects: gross morphology, hypoplastic defects of the molars, canines and incisors, as well as enamel thickness of the upper first and second molars.
Results: The observed morphology of the first molars corresponds to the typical aspect of mulberry molars, while that of the canines is characterised by hypomineralisation. Hypoplastic grooves were observed on the incisal edges of all incisors. The enamel of the first molars is underdeveloped while in the second molars a thick-enamelled condition is observed.
Conclusions: Our observations for the dental and skeletal evidence are supportive to a diagnosis of congenital syphilis for this specimen from XVI century Croatia. The use of CT imaging helped documenting the diagnostic features and quantifying the effect of the dental stigmata on first molars.

1. Introduction

Syphilis is a chronic disease that belongs to the group of treponematoses caused by the spirochetal bacterium *Treponema pallidum*. Based on mode of transmission it can be divided into two types: acquired, transmitted through sexual contact, and congenital, transmitted transplacentally to the developing foetus by an infected mother (Ortner, 2003)

Dental anomalies in patients with congenital syphilis have long been the subject of research (Hutchinson, 1857; Hutchinson, 1858; Fournier, 1884; Bauer, 1931; Sarnat & Shaw, 1942; Bauer, 1944; Putkonen & Paatero, 1961; Bernfeld, 1971), but in general, affected dental specimens are rare findings in archaeological contexts (Hillson, Grigson, & Bond, 1998; Gaul & Grossschmidt, 2014). Dental signs of congenital syphilis include defects of permanent teeth or dental stigmata: Hutchinson's incisors, Fournier's canines, Moon's molars, mulberry molars (Bauer, 1944). All of them have their origin in the process of amelogenesis. Although amelogenesis is genetically controlled, it is very sensitive to different environmental disturbances (Souza et al., 2013). Treponemal infection of enamel organs damages or destroys the ameloblasts and disrupts the formation of tooth germs (Bauer, 1944). Burket (1937) considered also metaplasia and aplasia of the ameloblasts as possible conditions caused by the treponematosis. Inflammation around the tooth germ can alter enamel formation in two main ways (Bauer, 1944; Hillson et al., 1998): (a) as minor or moderate hypoplastic defects and (b) as hypoplastic disturbance with morphological distortions. The former can occur in many teeth and in any period of enamel formation resulting in non-specific hypoplastic defects, while the latter can occur due to stronger pressure on the early tooth bud and during tooth morphodifferentiation resulting in the alteration of its shape. The

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inflammation around the tooth germ caused by congenital syphilis decreases with time, so the hypoplastic abnormalities affect only specific teeth at characteristic locations.

Hypoplastic changes caused by congenital syphilis can be divided into three main groups. The first group includes hypoplastic changes with clear and well-defined morphological features that are specific to congenital syphilis. These are Hutchinson's incisors and Moon's molars and they should be considered pathognomonic of this disease (Hutchinson, 1857; Hutchinson, 1858; Fournier, 1884; Hillson et al., 1998; Moon, 1877; Pflüger, 1924; Molnar, 1971). The inflammation due to *T. pallidum* during the morphological differentiation of the permanent teeth causes a disturbance in the formation of the enamel–dentine junction (Sarnat & Schour, 1941), which results in a typical appearance of the affected teeth. However, Hutchinson's incisors and Moon's molars occur only in a low percentage of subjects affected by congenital syphilis (Putkonen & Paatero, 1961) and with various degree of expression (Fournier, 1884).

The second group of hypoplasy includes Fournier's canines and mulberry molars that occur mostly, but not uniquely in congenital syphilis (Fournier, 1884; Jacobi, Cook, Corruccini, & Handler, 1992; Hillson et al., 1998). Therefore, Fournier's canines and mulberry molars are not defined as pathognomonic to congenital syphilis even though highly suggestive of this condition (Gaul & Grossschmidt, 2014).

These two groups of dental defects present a distinct morphology and are exclusively or mostly found in association to congenital syphilis. Therefore, Hutchinson's incisors, Fournier's canines, Moon's molars and mulberry molars are widely known as dental stigmata (Condon, Becker, Condon, & Hoffman, 1994; Hillson et al., 1998). Importantly, the aforementioned dental stigmata are typical of permanent teeth, and manifestation on deciduous teeth were observed albeit more rarely than in permanent dentition (Fournier, 1884; Steinbock, 1976; Hutchinson & Richman, 2006). In such cases, circular grooves around the root and just below the crown of the second deciduous molars have been typically detected (Karnosh, 1926). Fournier (Fournier, 1884) mentioned also the possible occurrence of notched deciduous incisors.

Finally, the third group of hypoplastic defects collectively designated as general enamel hypoplasia can be caused by syphilis as well as other environmental factors (Curtin, 2005). They are widely used as non-specific health indicators reflecting general health, diet, and, more broadly, living conditions. While in congenital syphilis hypoplastic changes appear on the structures that calcify up to the first year of life, the disruptions causing general enamel hypoplasia often affect dental formation for a limited period of time (Putkonen, 1962, 1963). Hypoplastic defects may appear as linear grooves, depressions (especially labially) or pits visible on the outer surface. The enamel is hard but deficient in amount. Among the hypoplastic manifestations darker and greyish colour of the enamel should be taken into account. The presence of systemic disorders such as the anhidrotic form of hereditary ectodermal dysplasia, dental dysplasia, linear enamel hypoplasia and molar-incisor hypomineralization (MIH) might lead to a wrong diagnosis of congenital syphilis. However, if single manifestations may be misleading, the observations carried out on more teeth and the estimation of the time of appearance of the disturbance can be considered altogether for a correct diagnosis. Disturbances in amelogenesis due to congenital syphilis mostly appear around birth and decrease throughout time. This locates enamel defects on the first molars and permanent incisors whose crown mineralization starts around birth. Time and location specificity distinguishes hypoplastic defects in congenital syphilis from most other environmental disturbances that appear throughout a longer period of life.

Congenital syphilis in past populations is difficult to confidently diagnose due to several reasons. This is firstly affected by the 50% fatality rate of infected foetuses (Aufderheide, Rodríguez-Martín, 2011; Roberts & Manchester, 2005). Additionally, degree of skeletal and dental preservation, age at death, and recognition of specific pathological changes influence the diagnosis (Roberts & Manchester, 2005). Bone deformities and dental stigmata are currently the main way in which congenital syphilis can be diagnosed in archaeological human remains. Teeth are usually better preserved than bones therefore dental changes are more often used for diagnosis.

Frequency of congenital syphilis in archaeological populations is hard to estimate owing to several reasons. The first are high rates of foetal death caused by the infection. Secondly, infected children may not display any sign of the disease, especially on the skeleton. Thirdly, even if symptoms occur, dental stigmata are observed in only 30% of individuals with congenital syphilis (Aufderheide & Rodríguez-Martín, 2011; Larsen, 1997) Cook and Powell (2005) reported only 5.1% frequency of congenital syphilis in juveniles. The authors stated that the lesions used to identify the disease were less specific than those for adults. Rothschild and Rothschild (1997) concluded that only 5% of syphilitic children showed evidence of dental or osseous involvement. This rate is explained by quick remodelling of new bone that leaves no visible trace on the skeleton. In a study by Putkonen (1962), Hutchinson's incisors and Moon's molars were present, in 45% and 22%, respectively of children born with syphilis, compared to only 12% that showed skeletal evidence of the disease. However, during the life span, teeth can be lost owing to dental and periodontal diseases, and in any case, the dental stigmata can be obliterated by wear and attrition, which in hypoplastic teeth would occur even at a higher rate. Although they possess very specific morphological characteristics, syphilitic teeth are quite sporadic findings in archaeological samples. However, the analysis of human skeletal remains from the 16th century archaeological site Park Grič in Zagreb, Croatia revealed the presence of five individuals with skeletal changes indicative of syphilis (Mašić & Pantlik, 2008a,b). Additionally, individual from grave 55, thereafter PG55, had dental signs supportive of congenital syphilis (Vodanović, Lauc, Premužić, & Rajić Šikanjić, 2013; Lauc, Rajić Šikanjić, Premužić, Fornai, Mašić, & Vodanović, 2014).

The first aim of this paper is to provide a detailed description of PG55 dental remains and discuss the gross morphology and appearance of the affected teeth taking into account their outer enamel surface. Although it is known that the disturbance of the ameloblasts during the secretory phase may cause a reduction in enamel thickness (Souza et al., 2013) the enamel hypoplasia typical of teeth affected by congenital syphilis has been described in the literature only qualitatively, while no quantitative analyses have been performed up to now on syphilitic dental specimens. Also, little is known about enamel thickness and morphology of the dentine crown in symptomatic teeth of individuals affected by congenital syphilis. Therefore, we aim to investigate the 2D and 3D relative enamel thickness and dental tissue proportions in PG55 affected and unaffected maxillary molars in comparison to non-pathological molar teeth from recent modern humans.

2. Materials and methods

PG55 was a female aged 17–20 years at death based on morphological characteristics of the skeleton and dentition (Buikstra & Ubelaker, 1994; Scheuer & Black, 2004). The skeleton was very well preserved, with only some of the small bones and teeth missing owing to post-mortem damage (Fig. 1, right individual). Apart from the dental changes, there were additional lesions visible on the skeleton. Significant endocranial lesions were

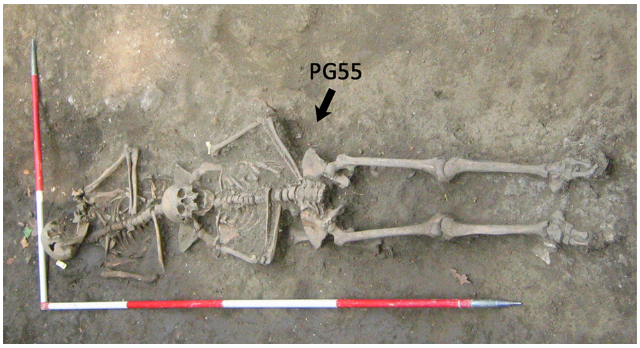

Fig. 1. Grave 55 in situ (right individual).

present on the frontal and on both parietals and occipital bones. The right orbital roof showed healed *cribra orbitalia.* Periosteal changes in the form of striation and woven bone were observed on both tibiae.

The analysed dental remains come from a fragmented maxilla and a very well preserved mandible (Figs. 2 and 3). Most of the upper teeth are present except for the left first incisor (LI^1) and the third molars (M^3s). In the lower jaw both fourth premolars (P_4s), the left first molar (LM_1), and the third molars are missing (M_3s). The right second deciduous molar is still present (Rdm_2).

The morphological description of the outer aspect of dental remains was carried out with the aid of a table lamp and Olympus SZX10 research stereo microscope.

Radiological examination of the specimen was first done by means of a CBCT scanner Scanora 3D, Soredex, Finland, (isotropic voxel size 250 μm) at Zubni rendgen Dr. Lauc Laboratory, Zagreb, Croatia. High resolution 3D image data of the dental remains were obtained at the Vienna micro-CT Laboratory, University of Vienna, Austria, through a Viscom X8060 micro-CT scanner, Hannover, Germany (130 kV, 100 mA) at an isotropic voxel size of 24 μm.

Of the teeth preserved, we considered the RM^1 (less worn than the LM^1) and both M^2s for the enamel thickness analysis and dental tissue proportions. Therefore, micro-CT data were produced also for a comparative recent *Homo sapiens* sample (12 M^1s and 20 M^2s; in four cases both M^1 and M^2 were from the same individual). These individuals were from different geographical areas, as detailed in Table 1. The teeth included in the study presented the same degree of wear as PG55 molars, namely, Molnar's stage 4 for the M^1s, and stage 1–2 for the M^2s and had a complete and well preserved crown. The image data for all teeth considered were segmented using the software package Amira 5.5 (FEI, http://www.vsg3d.com/) and following the indications by Spoor, Zonneveld, & Macho (1993), in order to virtually separate the dental tissues and generate 3D surface models of enamel and dentine. The 3D analysis of the enamel thickness for M^1s and M^2s was carried out following the protocol described in great detail in Benazzi et al. (2014). In particular, we used their protocol "3D-a" for molar teeth where the crown is separated from the roots at the cervical plane, namely the plane interpolating the cervical line of the tooth. According to Olejniczak et al. (2008) the following measurements were gathered from the virtual model of each molar crown: the volume of the enamel cap (mm^3); the volume of the dentine core (including the coronal pulp—mm^3); the area of the enamel–dentine junction (EDJ; mm^2). These measurements were used for the computation of the 3D average enamel thickness index (3D AET = volume of enamel divided by the EDJ surface; index in mm). The 2D analysis of enamel thickness was carried out on mesial sections of the M^1 crowns, following Benazzi et al. (2014). This protocol consists in setting the cervical plane of the molar crown as for the 3D analysis which serves as a reference for identifying the mesial section using a plane perpendicular to the cervical plane and passing through the protocone and paracone horn tips. Since these guidelines are conceived for unworn teeth (or at least for teeth in which the dentine horn tips are not affected by wear), we had to establish a protocol for the identification of alternative points to horn tips through which the section should pass. Similarly to Fornai et al. (2014), using a spline curve (namely, a continuous and smooth curve) we prolonged virtually the occlusal ridges on the exposed dentine patch; the mid-point of the spline trait crossing the exposed dentine patch was used for the identification of the section (see Fornai et al., 2014 for more details).

Fig. 2. Upper dental arch.

Fig. 3. Lower dental arch.

From the virtual section of each molar crown the following values were gathered (Olejniczak et al., 2008): the area of the enamel cap (mm^2); the area of the dentine core (including the coronal pulp—mm^2); the length of the enamel–dentine junction (mm), for the computation of the 2D AET (enamel area divided by the EDJ length; index in mm).

3. Results

3.1. Description of the dental remains

The dental remains of PG55 are well preserved. Some of the missing teeth were lost post mortem (LI^1 and RP_3), while the LM_1 was lost ante mortem, since the alveolar bone showed clear signs of resorption. The P_4s and all four M3s were agenetic or aplastic, in fact no dental germs or non-erupted teeth were visible in the micro-CT images. The only tooth with carious lesion is the lower right second deciduous molar (Rdm_2).

Table 1
Enamel thickness components and relative tissue proportions (both in 3D and 2D) for the maxillary first molars (M^1s) and maxillary second molars (M^2s; in 3D). For the comparative sample the averages and standard deviations (in brackets) are reported.

	Specimens	Provenience and number of individuals	Wear stage (Molnar, 1971)	Enamel volume	Dentine (and pulp chamber) volume	EDJ area	3D AET	Dentine volume on total crown volume (%)
M^1 crowns	Park Grič 55, L	Croatia	4	118.04	223.64	172.61	0.68	65.45
	Park Grič 55, R	Croatia	5	86.37	206.96	139.89	0.62	70.56
	Comparative sample	Australia = 3 Central Europe = 3 Southern Africa = 6	4	195.98 (40.59)	268.11 (60.09)	193.75 (28.55)	1.01 (0.10)	57.70 (2.67)
M^2 crowns	Park Grič 55, L	Croatia	2	277.95	210.82	164.16	1.6931652	43.13276183
	Park Grič 55, R	Croatia	1	292.71	223.23	171.59	1.7058686	43.26665891
	Comparative sample	Australia = 3 Central Europe = 5 Eastern Africa = 1 Papua New Guinea = 4 Southern Africa = 6	1–2	234.05 (63.58)	233.39 (56.09)	173.68 (26.07)	1.34 (0.20)	50.05 (3.52)

	Specimens	Provenience and number of individuals	Wear stage (Molnar, 1971)	Enamel area	Dentine (and pulp chamber) area	EDJ length	2D AET	Dentine area on total crown area (%)
M^1 mesial sections	Park Grič 55, L	Croatia	4	11.20	38.77	18.30	0.61	77.59
	Park Grič 55, R	Croatia	5	12.42	37.05	17.52	0.71	74.89
	Comparative sample	Australia = 3 Central Europe = 3 Southern Africa = 6	4	14.57 (2.29)	36.28 (5.72)	16.97 (1.68)	0.86 (0.08)	71.32 (2.03)

EDJ = enamel-dentine junction; AET = average enamel thickness.

PG55 occlusal condition is described below according to the indications given in Corruccini (1999). On the right side the M^1 paracone overlaps with the M_1 buccal groove so the buccal segment relation (BSR) was in Angle Class I. On the left side sagittal discrepancy of BSR with distal displacement of the lower dental arch was noted on the canines (Angle Class II). On the left side posterior crossbite was found on the second molars (M2s). The RP^4 was rotated about 140° and upper and lower midline discrepancy was 3 mm. The PAR index was 25, which implies greater occlusal disbalance, partially due to the lack of both P_4s. Panoramic reconstruction from the 3D data is presented in Fig. 4.

3.1.1. First molars

All first molars (M1s) present significant enamel defects. The RM^1 is reduced in all crown dimensions with respect to the M^2s (Figs. 5 and 6). The RM^1 presents a Molnar's wear stage 4 (Molnar, 1971), but the tip of the paracone does not show any wear facets. This cusp is underdeveloped and much reduced in the bucco-lingual dimension presenting a peculiar spoon shape. The paracone is marked by deep grooves exposing small patches of dentine at the base of its lingual aspect. The paracone is characterised by multiple rounded rudimentary enamel nodules. In general, the whole occlusal enamel shows heavy hypoplastic defects in the form of deep grooves. The cusps forming the trigone are characterized by marked hypoplastic defects visible on the central fovea and along the trigone periphery. A hypoplastic ring of deep pits marks the dental crown deeply on all its lateral aspect along the occlusal third. The mesial side is heavily affected especially in correspondence to the paracone.

The LM^1 (Figs. 6 and 7) is heavily worn showing a Molnar's wear stage 5 (Molnar, 1971). It presents the same size of the M^2 at the base of the crown. The enamel that is still visible on the occlusal surface is highly hypoplastic. The enamel at the paracone is thin with infolded yellow hypoplastic nodule on the tip of the cusp. The paracone is characterized by hypoplastic pitting. The paracone presents a similar spoon shape as in the antimere but less pronounced. Hypoplastic defects are visible in the form of grooves and pits, especially at the palatal aspect of occlusal surface and at the central basin. A ring of hypoplastic pitting surrounds the crown at the base of the cusps and it is more visible on the mesial and palatal aspects than on the distal and buccal sides. On the palatal surface hypoplastic grooves outline protocone and hypocone to the first third of palatal surface and split to the mesial and distal side.

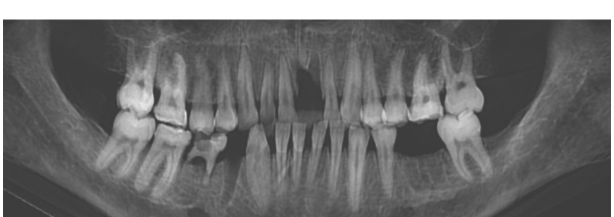

Fig. 4. Panoramic reconstruction from CBCT data.

Fig. 5. The first right upper molar (RM^1).

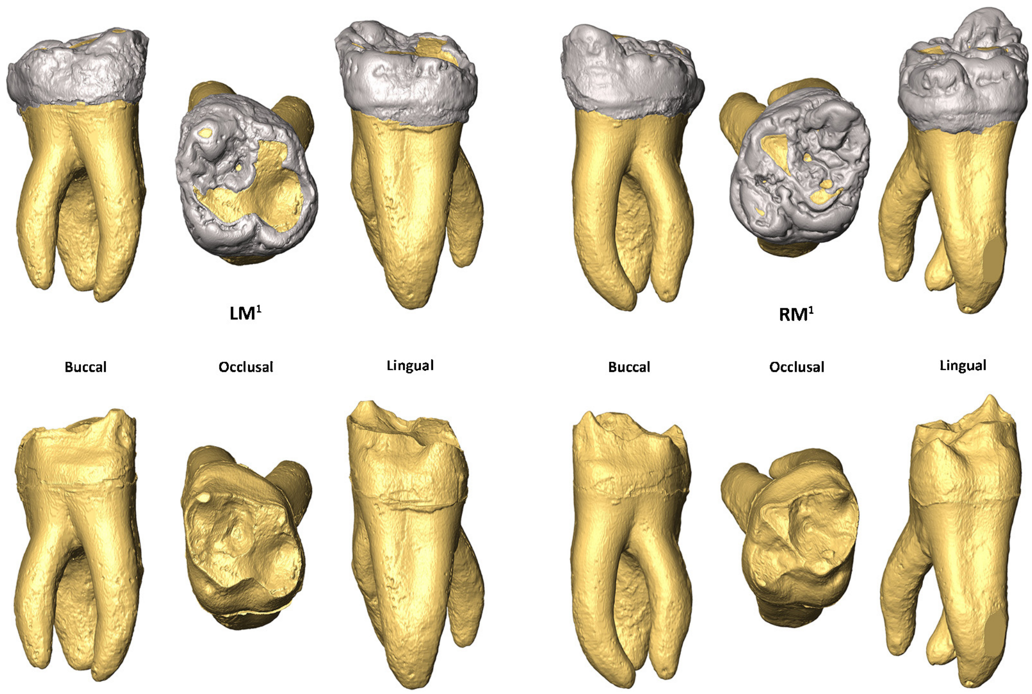

Fig. 6. The first left and right upper molars (LM^1 and RM^1).

The RM_1 (Figs. 8 and 9) is slightly smaller than the lower second molar. Even under the caveat of wear affecting this tooth (Molnar's wear stage 4 (Molnar, 1971), it still seemed to possess a rather flat occlusal aspect with markedly reduced cusps. The occlusal surface is characterised by deep grooves on all preserved occlusal enamel, and the central basin is highly crenulated. The thin enamel is infolded in all cusps. The outer surface shows multiple rounded enamel nodules on the occlusal surface and crests of enamel on a base of hypoplastic deposits. The lateral aspects of the crown are marked by a ring of grooves and pits. This is more evident on the mesial, distal and lingual sides and shallower on the buccal side.

3.1.2. Incisors

All incisors (Is) present are normal in size (Figs. 2 and 3), but show hypoplastic enamel alterations. They are worn to stage 4 of Molnar (Molnar, 1971). In spite of wear, it is possible to observe hypoplastic grooves marking the incisal edges of all Is. The upper Is show a rather smooth labial surface and irregular deep pitting on the lingual aspects. The labial aspect of the lower Is is rather smooth, but a very deep and large pit appears on both right I_1 and I_2. On the lingual side of the lower incisors, a sharp horizontal linear groove is present between the first and the second third of the crown possibly at the same developmental level up to the first year of life in all the Is.

Fig. 7. The first left upper molar (LM^1).

3.1.3. Canines

Upper and lower canines (Cs) are worn to Molnar's wear stage 3 (Molnar, 1971). Most of the hypoplastic defects in the Cs are located at the apical aspect of the crown. In spite of wear, sharp circular grooves are visible around the tips on both lingual and buccal surfaces of upper and lower Cs. The tip of the C cusp is partially hypoplastic in the form of circular defect around the tip of the crown. The upper Cs exhibited irregular pit hypoplastic defects on the buccal surfaces. Upper Cs exhibit no mesial canine ridge, distal accessory ridge or *tuberculum dentale*.

3.1.4. Premolars and second molars

All third and fourth premolars (P3s and P4s) as well as the M2s do not show any morphological or depositional deficiency. The RM^2 is the only second molar in this individual characterised by a paracone parastyle (stage 4 according to the ASUDAS). Although the left M_1 is absent, we can still observe that the M2s are visibly larger than the M1s.

3.1.5. Lower deciduous second molar

A large carious lesion obliterated almost completely the occlusal aspect of the dm_2 reaching the pulp chamber in its bucco-distal portion. Only the paraconid is still preserved and does not show signs of hypoplasia. The roots are well preserved in the cervical portion and do not present the circular hypoplastic groove described by Karnosh (1926).

Fig. 8. The first right lower molar (RM_1).

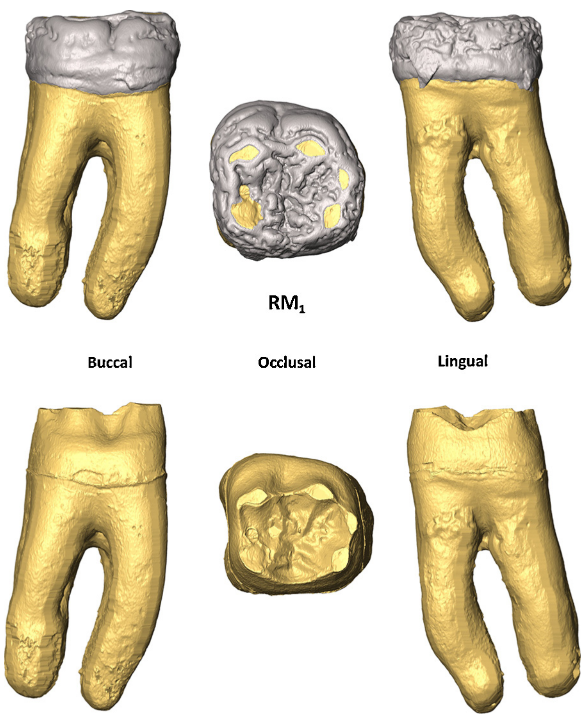

Fig. 9. The first right lower molar (RM_1).

3.2. 2D and 3D enamel thickness analyses and dental tissues proportions

The results of the 2D and 3D enamel thickness analyses for M^1s and M^2s are shown in Table 1 and visualised by means of box plots in Figs. 10–13. The results of the 3D relative tissue proportions analysis are shown in Fig. 14. The mesial sections of PG55 right and left M^1s and the results for the M^1 dental tissue proportions are shown in Fig. 15.

The RM^1 delivered low values of the enamel thickness components. Dentine volume and EDJ surface are among the lowest values of the sample, but the enamel volume is the lowest at all, and falls outside the range of variability of the comparative sample considered. Hence, the 3D AET for PG55 RM^1 is the lowest scored, and is outside the range of variability for the comparative sample.

On the contrary, both PG55 right and left M^2 have the highest enamel volume spread on a rather small dentine crown. As a result, both PG55 M^2s provide the highest 3D AET values within the sample considered.

As it can be observed on the mesial sections in Fig. 14, PG55 M^1s present defective enamel on both sides (on the middle and occlusal thirds), but especially on the occlusal area, in the portion of the section crossing the central fovea. The dentine portion of the section does not show alterations. PG 55 RM^1 provided the lowest 2D AET value within our sample (Fig. 16).

4. Discussion

Among 180 individuals from the 16th century cemetery Park Grič, Zagreb, Croatia, five of them have been reliably diagnosed with syphilis based on skeletal changes (Premužić, Rajić Šikanjić, & Mašić, 2012). The individual from grave 55, analysed in this study, is the only one presenting dental changes supportive of congenital syphilis. Since syphilis spread throughout Europe in the 16th century and had all the characteristics of an epidemic, it can be expected that the number of syphilitic individuals recovered at this site reflect the morbidity of syphilis in the urban populations of that time. The ratio of 6/180 is in accordance to 3–4% of syphilis morbidity for the 16th century populations in Central Europe (Sabbatani, 2006).

We analysed the dental remains of PG55 focusing on three aspects, namely (1) gross morphology of the M1s, (2) hypoplastic defects of the M1s, Cs and Is, (3) enamel thickness of the M^1s in comparison to that of M^2s, and integrated them all. In addition, we discussed observed skeletal lesions.

A confident diagnosis of congenital syphilis should be based on pathognomonic manifestations (i.e. Moon's molars or Hutchinson's incisors), which are however not ubiquitous (Putkonen & Paatero, 1961; Cruickshank, 1939). Alternatively a molecular

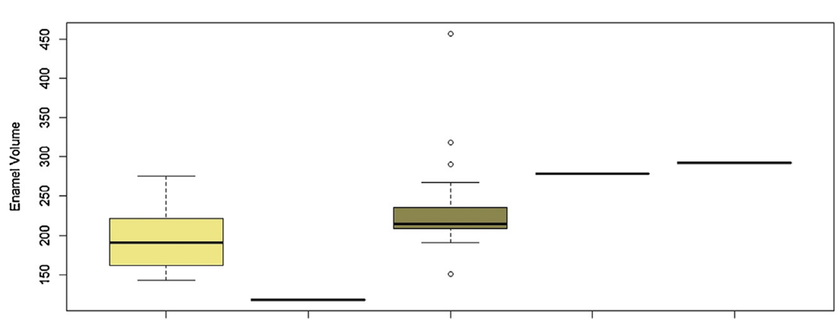

Fig. 10. Box plots for the enamel volume for PG55 left M^1 (PG55LM1) and PG55 left and right M^2s (PG55LM2 and PG55RM2) compared to the non-pathological M^1 and M^2 samples (UM1s and UM2s).

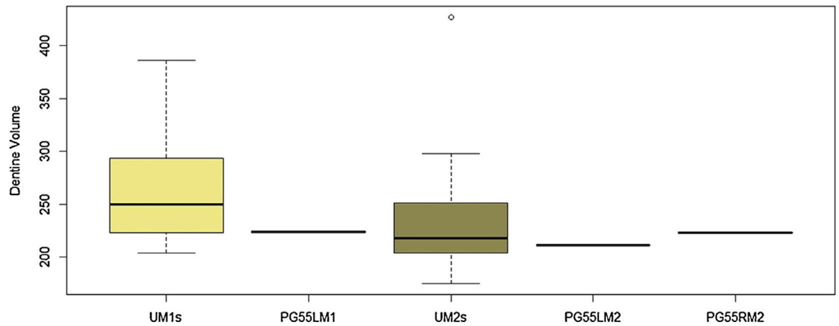

Fig. 11. Box plots for the dentine volume for PG55 left M^1 (PG55LM1) and PG55 left and right M^2s (PG55LM2 and PG55RM2) compared to the non-pathological M^1 and M^2 samples (UM1s and UM2s).

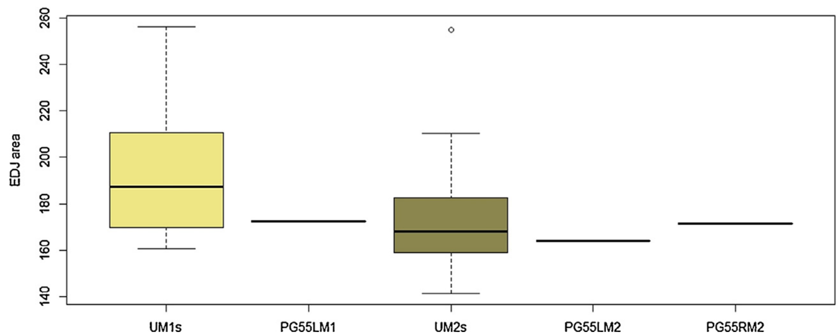

Fig. 12. Box plots for the enamel dentine junction (EDJ) area for PG55 left M^1 (PG55LM1) and PG55 left and right M^2s (PG55LM2 and PG55RM2) compared to the non-pathological M^1 and M^2 samples (UM1s and UM2s).

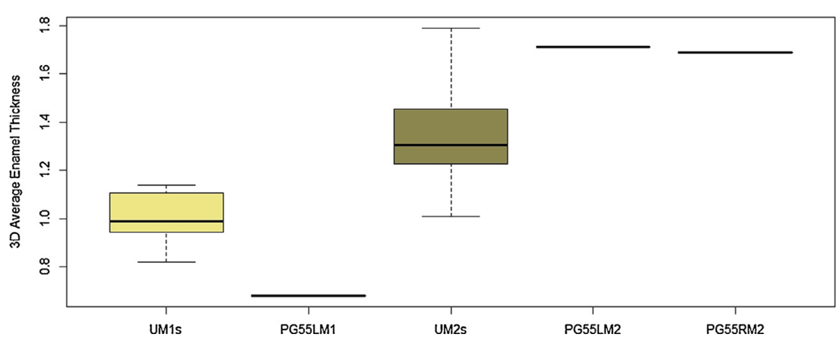

Fig. 13. Box plots for the 3D average enamel thickness (3D AET) for PG55 left M^1 (PG55LM1) and PG55 left and right M^2s (PG55LM2 and PG55RM2) compared to the non-pathological M^1 and M^2 samples (UM1s and UM2s).

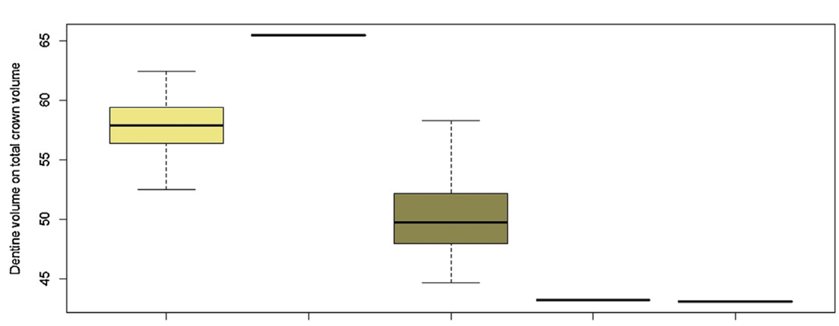

Fig. 14. Box plots for the proportion of the dentine volume (including the pulp chamber) on the total crown volume. PG55 right M^1 (PG55RM1) and M^2s (PG55M2s) are compared to the rest of the M^1 and M^2 non pathological samples.

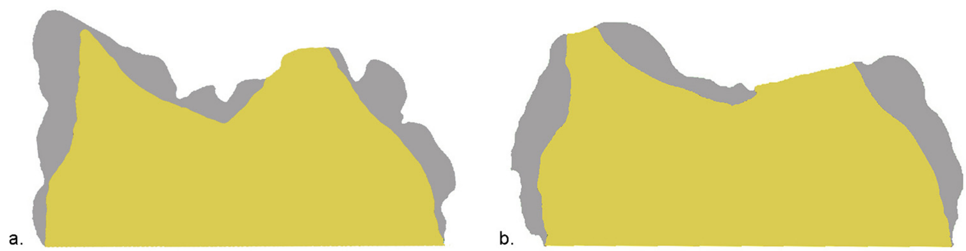

Fig. 15. Mesial sections from PG55 right (a) and left (b) M[1]s. The left section was flipped horizontally to ease visual comparison. Only PG55 RM[1] was included in the 2D AET analysis.

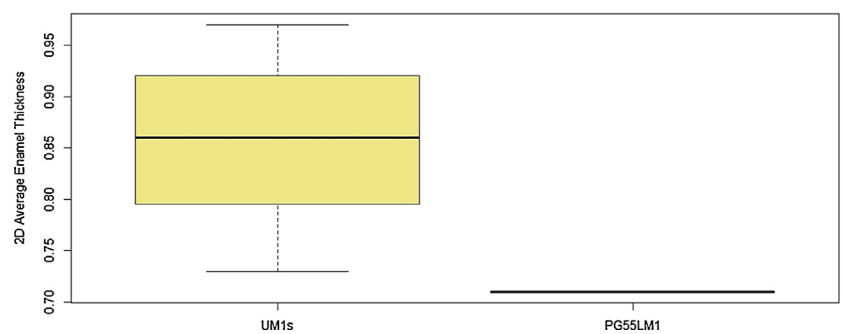

Fig. 16. Box plots for the 2D average enamel thickness for PG55 right M[1] (PG55RM1) and the non-pathological M[1] sample (UM1s).

detection of treponemal DNA should be carried out, but this procedure is destructive and not yet standardized for archaeological cases (Gaul & Grossschmidt, 2014).

In archaeological settings, strong support for congenital syphilis comes from other dental manifestations such as mulberry molars described by Fournier (1884) and regarded by Jacobi et al. (1992) and Mayes, Melmed, & Barber (2009) as distinctive signs, especially in pre-antibiotic cases. A circular groove around the C' tip (also described by Fournier (1884)) is interpreted by some as one of the possible manifestations of congenital syphilis (Putkonen, 1962; Cruickshank, 1939; Colyer, 1923; Bradlaw, 1953). Non-specific enamel hypoplasia is as well indicative of growth disruption associated to congenital syphilis and affecting dental morphogenesis up to the second year of life (Steinbock, 1976; Karnosh, 1926; De Wilde, 1943). In addition, particular skeletal changes are supportive of congenital syphilis if taken in conjunction to specific dental manifestations (Ortner,2003; Steinbock, 1976).

Hereafter, we consider observed dental and skeletal changes in the context of presenting a diagnosis of congenital syphilis in individual from Park Grič.

4.1. Mulberry molars

PG55 M1s are characterised by multiple rounded rudimentary enamel cusps and nodules of the enamel surrounding the cusps with agglomeration of masses of globules giving them the appearance of a mulberry. The plane-form hypoplastic defect affecting the basal area of the M1s' cusps corresponds to the classical aspect of a mulberry molar and distinguishes them from cuspal enamel hypoplasia as described by Ogden, Pinhasi, & White (2007). Importantly, all of the three M1s in PG55 present the same pathological aspect.

Karnosh's description of mulberry molars fits well to PG55 M1s: "Occlusal surface is rough, pigmented and pinched, with the exception of two or three nodules of good enamel huddled together which represents the poorly developed cusps. All around this occlusal deformity, the enamel of sound formation wells out in the form of a broad collar to the normal size of the crown at the neck of the tooth. [...] The true mulberry molar of syphilis is a first permanent molar characterized by enamel cusps showing crests of sound enamel on a base of hypoplastic deposits. These cusps are generally crowded together on a crown surface of dwarfed dimensions" (Karnosh, 1926).

The condition seen in PG55 cannot be attributed only to minor hypoplastic defects and consecutive tooth wear. PG55 M1s would be hardly worn away to that extent before adolescence, if the enamel had developed properly and fully, especially considering that in the same individual the M2s are only slightly worn.

Mulberry molars have been described in other cases of congenital syphilis from archaeological sites. In particular, we can directly compare PG55 M1s to the specimens shown in Mayes et al. (2009),Nystrom (2011) and Gaul & Grossschmidt (2014) despite the different degree of dental wear of these teeth, while we are not able to compare PG55 M1s to the putative mulberry molar shown in Erdal (2006).

4.2. General enamel hypoplasia

PG55 presents general hypoplasia clearly expressed on the Is, Cs and M1s in the form of linear grooves (Is), circular groove (Cs) and a combination of grooves and pitting in the occlusal third of the M1 crowns.

While the mulberry defects are mostly located on aspects of the crown that develop up to the first year of life, this generalized hypoplastic defects formed later and possibly simultaneously at an age ranging between about 1 and 1.6 years of age (according to the mean estimate of enamel formation by Reid & Dean (2006). Importantly, teeth that form later, i.e. premolars and second molars do not present any enamel alteration.

Putkonen (1962, 1963) stated that in congenital syphilis the hypoplastic changes appear on the structures that calcify up to the first year of life, and Hutchinson's incisors originate in the first three months after birth. According to Stones (1951), instead, the treponematosis might affect dental morphogenesis up to the age of two. Whether these hypoplastic changes must be considered as a response to the primary infection acting on the tooth bud, or as a result of a second disturbance might be debated. In any case congenital syphilis is generally regarded as a factor increasing severity of enamel hypoplasia, since affected children usually suffer with concurrent nutritional disorders (Jacobi et al., 1992; Steinbock, 1976; Karnosh, 1926; De Wilde, 1943).

In particular, the diagnostic value of the circular grooves marking the Cs' tips, usually defined as Fournier's canines[4], has been variously interpreted by different authors. For example, some authors (Hillson et al., 1998; Colyer, 1923; Bradlaw, 1953; Stoll, 1921) listed it as one of the dental stigmata typical of congenital syphilis, while others (Sarnat & Shaw, 1942; Putkonen, 1962; Cruickshank, 1939) considered this feature of scarce or none diagnostic value. Whether of the two views is correct, the same kind of enamel alteration has been described in several reports of congenital syphilis in archaeological settings (Gaul & Grossschmidt, 2014; Mayes et al., 2009; Nystrom, 2011).

4.3. Skeletal changes

The systemic inflammation occurring in congenital syphilis might cause different kinds of lesions. These might be specific, such as sabre shin tibia appearing also in acquired syphilis. However, most changes are general and unspecific, including periostitis, osteitis, and metaphyseal changes Aufderheide & Rodríguez-Martín, 2011; Rothschild & Rothschild, 1997; Rasool & Govender, 1989).

The analysed individual presents several cranial and postcranial lesions. Endocranial lesions can be observed on all cranial bones. On frontal and both parietal bones they appear as capillary changes, while on the occipital bone 'hair-on-end' lesions are visible. They may be caused by inflammation and/or haemorrhage of the meningeal vessels. These conditions can be the result of several diseases, such as chronic meningitis, trauma, metabolic disorders and tuberculosis (Lewis, 2007). Meningitis can be caused by syphilis, but in those cases the base of the skull is usually affected, causing hydrocephalus and distension of the scalp veins, and spastic paralysis, mental defects, fits and pituitary disorders can occur (Lewis, 2007). Research of possible cases of congenital syphilis by Cook and Buikstra associated presence of these lesions with periostitis, dental defects and a lower expectation of life (Cook & Buikstra, 1979).

Localized periostitis visible as striation and woven bone is observed on both tibiae. Periostitis is new bone deposition that forms as a result of injury of infection (Lewis, 2007). Rothschild & Rothschild (1997) stated that new bone formation can appear in various forms of treponemal diseases and but is not specific only for congenital syphilis. In congenital syphilis periostitis appears, although rarely, and also remodels quickly often leaving no visible signs (Rothschild & Rothschild, 1997).

In conclusion to the presence of observed skeletal lesions, it has to be stressed that only 5% of children with syphilis will exhibit skeletal or dental changes (Rothschild & Rothschild, 1997). Also, Cook & Powell (2005) verified that the lesions used in diagnosing syphilis in children were less specific than in adults.

4.4. Additional evidence

Here we include further occurrences which are not typical of congenital syphilis, but are either found in association to it or are otherwise supportive of this diagnosis. In particular, this individual shows a mild malocclusion consisting in a cross-bite. Malocclusion, although not characteristic of congenital syphilis, occurs frequently in affected individuals (Stathers, 1942).

The total of the morphological evidence described in PG55 can be coupled with the presence of syphilis at site, as testified by other skeletal remains showing distinct signs of acquired syphilis, to corroborate a diagnosis of congenital syphilis (Premužić et al., 2012).

4.5. Alternative diagnosis

The most probable aetiology for the enamel defects observed in PG55, can be determined by evaluating the type and expression of defects, their location, and estimating time of appearance of the disturbance. The time of appearance of the hypoplastic changes in conjunction with the fact that Ps and M2s are unaltered made us exclude many conditions that mimic congenital syphilis in their manifestations in the face and mouth of living persons such as the anhidrotic form of hereditary ectodermal dysplasia or dental dysplasia with ectodermal involvement. Gargoylism and achondroplasia that can also have similar manifestations were rejected due to lack of skeletal abnormalities typical for these disorders. Dental defects, as the non-specific health indicators can be seen as different hypoplastic forms, among which the linear enamel hypoplasia, furrow shaped defects, darker and greyish colour, liableness for decay, depression on the labial surface and MIH should be taken into account. Linear enamel hypoplasia and furrow shaped defects cannot explain specific morphological feature of the first permanent molars and difference between enamel thickness of first and second molars. As previously noticed, none of the present permanent teeth in PG55 have carious lesions and all of them show a normal colour. As expected, M1s present a higher degree of wear compared to M2s. However, it can be assumed that the presence of thinner and irregular occlusal enamel can cause the M1s to wear down at a faster rate. In fact, M1s present a degree of wear ranging from 4 to 5 (Molnar, 1971), while M2s are unworn or slightly worn. In MIH the enamel defects are usually asymmetric with well-demarcated white/opaque, yellow or brown discoloration affecting cuspal areas and sparing the cervical areas. In many cases the incisor enamel is affected, but often minimally (Farah, Monk, Swain, & Drummond, 2010).

Fagrell, Salmon, Melin, & Noren (2013) examined ground radial and sagittal sections from teeth diagnosed with MIH using the same method as in this study, X-ray micro-computed tomography, to estimate the onset and timing of the MIH and to relate the hypomineralised enamel to the incremental lines. Their findings indicate that the ameloblasts in the MIH are capable of forming enamel of normal thickness, but with a substantial reduction of their capacity for maturation of enamel (Fagrell et al., 2013). In our specimen the enamel thickness of M1 is significantly thinner and this condition cannot be attributed to MIH. The assumption that the MIH could explain these findings of the enamel defects was therefore rejected.

4.6. Enamel thickness

The enamel thickness components for PG55 M^1s and M^2s show an interesting pattern, since for both tooth types the dentine crown is rather small with respect to the comparative sample, but the enamel cap is absolutely and relatively scarce in the mulberry M^1s while the M^2s have a thick enamel cap. The M^1s' enamel underdevelopment finds an immediate explanation in the treponemal infection that in this individual must have acted on the dental germs perinatally (Bauer, 1944). Obviously, the conditions that gave origin to the mulberry M^1s had ceased at the time of M^2s crown formation; nonetheless, the thick-enamelled condition observed in M^2s is rather surprising. A possible explanation for it could be found in the context of the inhibitory cascade model (Kavanagh, Evans, & Jernvall, 2007). Owing to M1s' abnormal development, the M2s would have activated earlier resulting in big-crowned teeth. The dentine crowns of the molars seem to not be affected and present a normal morphology. Although wear affects the dentinal crown at the incisal edges of the Is and in lower degree those of the Cs, such observation can be done for the anterior teeth too.

5. Conclusions

The individual from grave 55 buried at the 16th century cemetery Park Grič in Zagreb, Croatia shows several dental and skeletal manifestations strongly supportive of congenital syphilis. The diagnosis is supported by the following: (1) mulberry molars; (2) enamel hypoplasia marking the lingual aspect of the Is, the Cs' tips, and the occlusal third of the M1s, and possibly formed simultaneously; (3) endocranial lesions and tibial periostitis; (4) drastically reduced enamel thickness at the M1s, and normal M2s; (5) presence of malocclusion of M2s; (6) presence of acquired syphilis in several other individuals from the cemetery (Premužić et al., 2012). The diagnosis of congenital syphilis in this individual would make it the oldest case of this disease in the post-Colombian Europe (Gaul & Grossschmidt, 2014; Harper, Zuckerman, Harper, Kingston, & Armelagos, 2011).

Since teeth affected by congenital syphilis are relatively rare in the archaeological records, this individual is of particular relevance for the understanding of the variability in the expression of the symptoms of this pathology and the influence it might have on dental development (Rothschild & Rothschild, 1997; Harper et al., 2011).

The CT images are a suitable means for the analysis of dental remains and in cases of congenital syphilis can inform about the hidden changes on dentine incisal edge under the enamel cap. The analysis of the enamel thickness and dental tissue proportions we carried out on upper first and second molars is the first qualitative assessment of this sort on a individual affected by congenital syphilis. It provided more insight into development of enamel in cases of congenital syphilis and might serve as comparative reference for future studies.

Conflict of interest

There are no conflicts of interest to declare.

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