

To which extent does the geo-chemical composition affect enamel susceptibility to carious lesions? A chemical analysis using LA-ICP-M

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Introduction

Carious lesions are one of the most common infectious diseases that affect humankind. They became endemic after humans switched from hunter-gathering to an agricultural production economy. One of the main reasons for the insurgence of carious lesions is diet; specifically, a diet high in carbohydrates facilitates the development of the dental plaque formed by the bacterial fauna that commensally lives in the oral cavity. Though the formative process of carious lesions is associated to the commensal bacteria in the oral cavity, whose acidic waste by-products demineralize the dental tissue, this infectious disease is under the influence of many intrinsic and extrinsic factors, ranging from diet, daily habits in food preparation, access to resources, social

status, hormonal fluctuations in relation to pregnancies that predispose women to higher levels of carious lesions, and oral hygiene (1-4).

One of the extrinsic, environmental factors, that play a role in the development of caries, is the presence of fluoride in drinking water. Fluoride is well known to be an element with buffering effects on the insurgence of carious lesions. This chemical element can be found as part of the geological composition of the environment or can be intentionally added to drinking water through fluorination; its incorporation into the enamel during amelogenesis or in subsequent phases of post-eruptive mineralization of the enamel cup has proven to prevent the insurgence of carious lesions (5, 6). However, chemical studies have also shown that trace elements such as Ba, Li, Mo and Sr correlate to low frequencies of caries, while a positive correlation was found for Cu and Pb (7).

Various studies have shown some discordant results in the effects that water and soil composition have on carious lesions. The present study analyzes the chemical composition of human dental enamel by mean of LA-ICP-MS (laser-ablation inductively-coupled-plasma mass-spectrometry) in three Maya Prehispanic and colonial sites in the Yucatán Peninsula characterized by different hydro-geological composition (Table 1 and Figure 1). The trace-element composition is then compared to the rates of carious lesions recorded in the adult segment of the populations in order to assess to which extent the geochemical composition acts to buffer, or facilitate, the insurgence of caries.

Materials and Methods

The trace elemental concentration of each site was established by analyzing the chemical composition of the first permanent molar in infant and juvenile individuals from approximate age 3 to 8 years old, who are supposed to have been born and grown up in the region and, therefore, should bear the chemical signature of the place (8). Moreover, the direct analysis of human enamel informs about the real incorporation of trace elements in this tissue, dribbling the potential source of error represented by biopurification. Trace elements were measured by means of LA-ICP-MS at the IIRMES laboratories at California State University, Long Beach.

Carious lesions were scored on all permanent teeth of the adult individuals (Figure 2). The skeletal collection from the site of Xcambó could be divided into Early Classic and Late Classic, AD 250-550 and AD 550-700 respectively, (4); Calakmul belongs to the Classic period, AD 300-800, (9) and Campeche collection comes from the early colonial downtown cemetery, AD 1,540-1,650, (10).

Results

Trace element composition discriminates the three sites mainly on the base of Sr and Ba concentrations (Figure 3). Logarithmic values indicate higher concentration of Sr at Xcambó, less at Campeche (both showing overlapping ranges of variability for Ba) and higher concentrations of Ba for Calakmul. All the other trace elements in the range of atomic weight between 23 (Na) and 238 (U) do not contribute to discriminate among the sites, in particular Mg and Zn.

Frequency of carious lesions can be appreciated in Table 2, divided by sex and period for Xcambó (4), by sex and social status for Calakmul (9) and by ethnicity for Campeche (10); the latter could not be scored by sex due to the state of preservation of the remains.

As regards Xcambó, the sample from the Early Classic period consists of 23 individuals, ten being males and 13 females. On the contrary, 66 females and 71 males form the Late Classic period collection. Differences in the male sub-groups between Early Classic and Late Classic are statistically significant, in particular for the posterior and total dentition. On the contrary, the difference for the female cohort at this coastal site in northern Yucatan does not fully reach the level of significance between periods ($p=0.066$), even though such values is close to significance. Within-period differences between sexes are significant for both the Early and Late Classic. An analysis of age at death composition in the various male and female sub-groups indicates that age is not a factor in the different frequency of carious lesions between periods or within periods.

The male and female sample from Calakmul is divided by social status (no specific chronological horizons within the Classic period could be distinguished). In this case, high-status males present the lowest rate of carious lesions (only 1.4% of teeth affected) in comparison with high-status females 8.8% and low status males and females (respectively 6.3% and 6.9%). Such difference is statistically significant ($p=0.024$), while among high status females and male and female low status groups differences are not significant ($p=0.719$). Also in this case, age at death is not a factor in the rate of lesions.

Finally, the Campeche sample is divided in "natives" and "mestizos". Such difference rests on the pattern of morphological dental traits (10). Individuals were assigned to the "mestizo" group when the pattern of dental morphological traits did not correspond to that of Prehispanic Maya populations (in such case they were considered natives), nor could be assigned to Africans or Europeans that also formed part of the cemetery sample. In this case, due to the poor state of preservation, samples could not be analyzed by sex. Frequencies of carious lesions clearly separate natives, with more than 22% of their teeth affected, from mestizos which present less than 10% of their teeth with caries. In the case of natives and mestizos, Price and Burton (11) show that all of them are local, by

showing Sr87/86 values that fall within the range of variability of Campeche.

Discussion

Cariou lesions are multifactorial in origin. Diet, daily habit, physiological factors, pregnancies, oral hygiene, all play a role in the insurgence of such infectious disease (1-4). As Hildebolt (5) pointed to, also the chemical components of the environment can protect from the insurgence of caries. Fluoride, either naturally present or intentionally added to drinking water through fluorination, has proved to decrease the risk of individuals being affected by carious infections (12).

The prevalence of carious lesions was noted to vary among geographical regions and the local concentration of many trace elements was associated to a higher or lower prevalence of the oral infectious disease. Curzon (7) and Curzon and Crocker (13) reported that elements such as Sr, Ba, V, Mo, Mn, Al, Ti, and P, when present in high concentrations in the whole enamel, were associated with low caries (i.e. they are cariostatic), while Pb, Cu, Cr, Zn and Se were positively associated to them. In turn, Navia (14) suggested that elements like Ba, Al, Ni, Fe, Pd and Ti have no influence on caries (15). Similarly, Vrbic and Stupar (16) found a correlation to dental caries for Sr and Al. However, not all the elements in the enamel were found to be directly correlated to their concentration in the soil (17), so that also the association between elements and carious lesions may not be fully supported.

In the present study, with the exception of Na, Ca, Mn, Mg, Zn, Ba, Sr and P, all the elements between atomic weight 23 and 280 were below LA-ICP-MS' detection level. On the contrary, fluoride lays outside of the range of elements that can be analyzed by means of laser ablation ICP-MS. The three sites examined in this study are discriminated only by Sr and Ba, a result similar to what found by Curzon and Losee (18) for the Eastern United States. In particular, strontium concentration in bones and teeth has often been reported as directly associated to the environment (19). With respect to their association with oral infectious diseases, strontium should theoretically provide chemical protection to teeth from the development of carious lesions (7,14), while discordant opinions arise concerning the properties that barium may have with respect to the insurgence of caries.

The frequency of lesions in the three samples investigated in this study is very variable, among and within collections. At Xcambó, a significant increase in caries occurred between the Early and the Late Classic. Males increased frequency from 7.4% to 14%, while females from 21.2% to 27.4%. However, Cucina et al. (8) did not note consistent difference in the chemical composition of the adult segment of this coastal population between the two periods. In fact, while all the Early Classic

adults investigated by LA-ICP-MS fall within the range of variability of the local infants, approximately only 10% of the Late Classic adults fall outside such range which means that the vast majority of adults during the Late Classic were local.

The values from Calakmul (without the high status male cohort) are similar to those of male individuals at Xcambó during the Early Classic, even though the two sites are discriminated by Ba but not by Sr. Similarly, Campeche presents highly variable data, with the natives showing frequency as high as in Xcambo's females, while "mestizos" showed a frequency of caries that do not differ significantly from Xcambo's Early Classic males and Calakmul's. Obviously, the poor state of preservation of the Campeche sample limits the possibility to assess the effects of sex and age-at-death on the frequency of carious lesions. Nonetheless, a general demographic profile of the Campeche skeletal sample indicates that the majority of the adult individuals died before age 40 (20).

Apparently, trace element composition does not provide a solid base to protect individuals from the insurgence of carious lesions. Most of the previous works discussed the presence of multiple elements in single sites instead of comparing rates of caries among sites based on elemental concentrations. In turn, even though Curzon and Losee (18) found that higher concentration of Sr was associated to a lower frequency of caries in the comparison between high-caries and low-caries samples in Eastern United States, the same authors (21) could not replicate such positive correlation when they analyzed similar samples in the Western United States.

I do not rule out that alongside fluoride, other elements can interfere with caries; the presence of high concentrations of Sr seems to reduce acid dissolution of hydroxyapatite. The case of Calakmul, that shows lower concentrations of Sr in comparison with Xcambó and overall lower rates of carious lesions than Xcambó, and the case of Xcambó itself, with identical environmental conditions between the Early Classic and the Late Classic but with significantly different rates of carious lesions between periods, seem to clearly indicate that other factors thoroughly described in the literature (diet, habits, sex, oral hygiene) prevail over enamel chemistry in preventing or favoring the development of this oral infectious disease.

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	Location	Period and sample composition
Xcambó	Northern Coast of Yucatan	Ealy (AD 250-550) and Late Classic (AD 550-700); males and females
Calakmul	Inland, Northern Peten	Classic period (AD 300-800); males and females; high and low social status
Campeche	Gulf of Mexico, Western Coast of Yucatan	Colonial (AD 1540-1650); natives and mestizos

Table 1. Location, chronology and composition of the samples analyzed

		Early Classic	Late Classic
Xcambó	Males	7.4	14
	Females	21.2	27.4
Campeche	Natives	22.8	9.7
	Mestizos		
Calakmul	High Status		
	Low Status		
	Males	1.4	6.3
	Females	6.9	8.8

Table 2.– Percent frequency of carious lesions in the three samples analyzed, by chronology, sex and social status.



Figure 1. Geographical map of the location of the three sites in the Yucatan Peninsula.



Figure 2. Carious lesion from Xcambó.

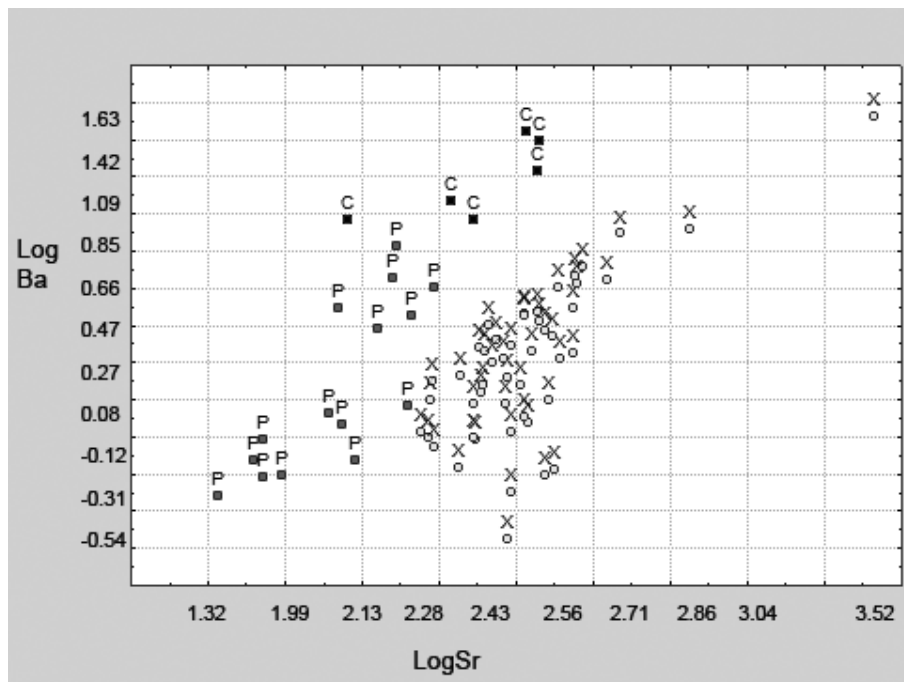


Figure 3. Scatterplot showing the distribution of log-values of Sr and Ba in the three samples.