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Dental age is an independent marker of biological age

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Abstract

Background Biological age markers are a crucial indicator of whether children are decelerated in growth tempo. Skeletal maturation is the standard measure, yet it relies on exposing children to x-radiation. Dental eruption is a potential, but highly debated, radiationfree alternative.

Objectives We assess the interrelationship between dental eruption and other maturational markers. We hypothesize that dental age correlates with body height and skeletal age. We further evaluate how the three different variables behave in cohorts from differing social backgrounds.

Sample and Method Dental, skeletal and height data from the 1970s to 1990s from Guatemalan boys were converted into standard deviation scores, using external references for each measurement. The boys, aged between 7 and 12, derived from different social backgrounds (middle SES (N = 6529), low-middle SES (N = 736), low SES Ladino (N = 3653) and low SES Maya (N = 4587).

Results Dental age shows only a weak correlation with skeletal age (0.18) and height (0.2). The distinction between cohorts differs according to each of the three measurements. All cohorts differ significantly in height. In skeletal maturation, the middle SES cohort is significantly advanced compared to all other cohorts. The periodically malnourished cohorts of low SES Mayas and Ladinos are significantly delayed in dental maturation compared to the well-nourished low-middle and middle class Ladino children.

Conclusion Dental development is an independent system that is regulated by mechanisms different to skeletal development and growth. Tooth eruption is sensitive to nutritional status, whereas skeletal age is more sensitive to socioeconomic background.

Take home message for students Dental eruption is an independent biological maturation system that is regulated by other mechanisms than body height and skeletal age. Dental eruption is sensitive to malnutrition and may serve as an additional tool to differentiate between malnutrition and other reasons for impaired growth in children.

Introduction

Children are defined as stunted if their height-for-age is more than two standard deviations below the WHO Child Growth Standards median (World Health Organization, 2015). Stunting is the impaired growth and development that children experience from poor nutrition, repeated infection, and inadequate psychosocial stimulation. Thus, height, weight, and body mass index are routinely used to classify the nutritional status of children in many Low and Middle Income Countries (LMIC). Poor nutrition does not only impair child growth, it can also delay bone maturation. For decades, it has been known that, irrespective of socioeconomic background, bone age of undernourished children is delayed; the more severe the undernutrition the more delayed the bone age (Alcázar et al., 1984). On the other hand, stunting is not a synonym of malnutrition (Scheffler et al., 2020). Being shorter than two standard deviations below the WHO's standard median is also a common feature among well-nourished and healthy children, and it is not always evident whether short stature results from food shortage, illness, inadequate psychosocial stimulation, or simply reflects a slower than average pace of growth and development. One of the most common causes of short stature is the benign idiopathic delay in developmental tempo ("late bloomer"), characterized by a substantial delay in bone age without any signs of impaired health (Aguilar and Castano, 2022; Creo and Schwenk, 2017).

Though it is well known that different parts of the human body grow at different rates and tempo (Prokopec, 2001), delays in developmental tempo are usually clinically diagnosed by the delay in bone maturation as the skeletal development is considered "the only means of assessing rates of maturational change throughout the growing period" (Cox, 1997).

However, assessing bone maturation requires exposing children to ionizing radiation, which poses a health risk (Meo et al., 2006). Additionally, as mobile x-ray apparatuses are expensive and difficult to transport due to their weight and size, they are not part of the standard equipment of anthropometric field work.

Counting the number of teeth that have erupted through the gums is an alternate anthropometric marker of developmental maturation. It is non-invasive and covers a relatively long period of growth, especially considering that two sets of teeth (deciduous and permanent) develop consecutively, three sets, if the third molars are included (Demirjian, 1986). In this study we are interested in the permanent teeth, which traditionally are said to occur between the ages of 6 and 13 years (Logan and Kronfeld, 1933).

However, tooth eruption as a marker of biological age, and the relationship between dental age and skeletal age, are under considerable debate. While some researchers argue that the two maturational processes have a strong positive correlation (Al-Balbeesi et al., 2018; Demisch and Wartmann, 1956; Liliequist and Lundberg, 1971; Sierra, 1987), Demirjian et al. (Demirjian et al., 1985), along with various other researchers (Beunen et al., 2006; Bielicki et al., 1984; Lewis, 1991), suggests, that tooth development is an independent system, as only weak or even insignificant correlations were found.

In the past, radiographic dental age assessment methods have been widely employed (Demirjian et al., 1973; Kumar et al., 2013; Nolla, 1960), but little research has been done using the non-invasive approach of dental eruption. There is a lack of dental age references, and practical methods to allow for transforming the state of teeth eruption into any useful developmental variable (Demirjian, 1986). Our main goal for this study is to assess the interrelationship of dental age, skeletal age and height using the number of erupted teeth as variable for dental age. To convert these three variables into comparable units, we used a z-transformation (standard deviation score = SDS). If the correlation between dental age SDS and skeletal age SDS is high, it would imply that they belong to dependent systems. We propose the following hypotheses:

1. Dental age SDS (dental SDS) shows a strong positive correlation with the skeletal age SDS (skeletal SDS).
2. Dental SDS shows a strong positive correlation with height SDS.
3. Skeletal SDS shows a strong positive correlation with height SDS.

The correlation of dental age towards other biological age markers should not be confused with a validation of the reliability of dental age as a biological age marker itself. To stress this, we additionally evaluate how dental age, skeletal age and height differ according to socioeconomic status (SES) and ethnicity in a second part. We assume that, if dental age enables us to significantly distinguish between cohorts based on developmental differences, it indicates a potential useful application of dental eruption as a non-invasive biological age marker, regardless of its relationship towards other developmental makers. We propose the following hypothesis:

4. Cohorts that differ significantly in skeletal age also differ significantly in dental age.

For the analysis, we are using a Guatemalan dataset that is comprised of three different social strata (middle, low to lower middle (low-middle), and low socio-economic status (SES)). The low SES cohort divides into a Maya and a Ladino group, whereas all other strata are Ladinos only. Both groups of the low SES cohort suffered from periods of malnutrition (Bogin and MacVean, 1981). While there is no such information about the low-middle SES cohort available, children of the middle SES group were not exposed to food insecurity.

Dental information was only available for boys, females could not be analyzed.

Sample and Method

We analyzed data from the Longitudinal Study of Child and Adolescent Development by the Universidad del Valle Guatemala (UVG). Between 1953 and 1999, various physical and cognitive variables were measured in Guatemalan school children of different social backgrounds and ethnicities. The two main goals of said study were firstly, understanding the processes of growth and development over time and secondly, to provide reference data for Guatemalan children. Back then, the regular use of x-radiation was not a concern, leading to sizable longitudinal and cross sectional datasets on skeletal maturation of Guatemalan children and adolescents of various social backgrounds (for more information see (Bogin and MacVean, 1983, 1984)).

We included observations of boys between the ages of 7- and 12-years with dental and/or skeletal information obtained between the early 1970s and the late 1990s (see Table 1). Some individuals were measured up to 4 (max 6) consecutive years, creating a mix of a longitudinal and cross-sectional data set. Dental information is given as the sum of permanent teeth which had at least perforated the gum with any part of the crown. This was examined by a clinical dentist. Skeletal information is given as bone age (Greulich and Pyle, 1959), using x-ray pictures of the left hand and wrist.

Table 1 Guatemalan boys with information on permanent teeth (dental data) and/or a skeletal age (skeletal data). Cohorts are separated by socioeconomic status (SES) and ethnicity.

SES	Ethnicity	Dental data N	Skeletal data N	Exposed to Malnutrition	School Fee
Middle	Ladino	4157	2540	No	Yes
Low-Middle	Ladino	716	90	Unknown	Yes
Low	Ladino	2285	1520	Yes	No
Low	Maya	4302	1219	Yes	No

For each child, we determined a "dental age", based on a Cuban reference that provided mean age and standard deviations for teeth eruption and was considered appropriate for Guatemalan children (San Miguel Pentón et al., 2011), and the "skeletal age" according to Greulich & Pyle. Dental age and skeletal age were transformed into z-scores,

$$\mathbf{Z} = \frac{x-\mu}{\sigma}$$

where x = individual age, μ = mean age of the reference when the respective individual state of dental and skeletal maturity was reached, σ = standard deviation of the reference). Height was transformed into z-scores based upon WHO-references. For the final analysis, we only considered children with maximum 27 teeth, since dental maturity scores for assessing the process of maturation are only meaningful if dentition has not been completed. Z-scores are referred to as SDS (standard deviation scores).

Statistics

The open source program RStudio (R version 4.0.2, R core team, 2020) was used for all analyzes.
To assess the interrelationship between height-, bone age- and dental age SDS, a correlation matrix (spearman) and linear models were implemented.
To assess differences between cohorts, requirements for parametric tests were checked as follows: normal distribution was tested using the Shapiro-Wilk-Test, followed by a visual verification with QQ-plots. The Levene-Test was used to test for homogeneity of variance.
Neither skeletal-, dental-, nor height SDS met the latter criteria. Dental z-scores did not follow a normal distribution. We used Kruskal-Wallis-Test for analyzing differences between groups. Pairwise comparison was done with the Dunn-Test, using the multiple comparison adjustment according to the Bonferroni method.

Results

Part 1: Correlation Between Height-, Skeletal- and Dental SDS

With r = 0.18, there is a significant (p = 0.00) but weak correlation between dental age SDS (dental SDS) and skeletal age SDS (skeletal SDS). The correlation between dental SDS and height SDS is also weak (r = 0.20, p = 0.00). Height and skeletal maturity show a moderate to high positive correlation (r = 0.58, p = 0.00).
The correlations per cohort are shown in Table 2. The low SES Ladinos show the weakest correlation between dental and height SDS (r = 0.16) and dental and skeletal SDS (r = 0.08), the latter being not significant. The low-middle SES Ladinos show

Table 2 Correlation matrix between height-, skeletal- and dental SDS per cohort (midLad = middle SES Ladinos, lowmidLad = low-middle SES Ladinos, lowLad = low SES Ladinos, lowMaya = low SES Maya).

Cohort		Height SDS	Skeletal SDS	Dental SDS
midLad	Height SDS	1		
	Skeletal SDS	0,56	1	
	Dental SDS	0,25	0,16	1
lowmidLad	Height SDS	1		
	Skeletal SDS	0,61	1	
	Dental SDS	0,32	0,29	1
lowLad	Height SDS	1		
	Skeletal SDS	0,66	1	
	Dental SDS	0,16	0,08	1
lowMaya	Height SDS	1		
	Skeletal SDS	0,57	1	
	Dental SDS	0,29	0,23	1

the strongest correlation between dental and height SDS ($r = 0.32$) and dental and skeletal SDS ($r = 0.29$). The correlation between height and skeletal SDS ranges between $r = 0.56$ (middle SES Ladinos) and $r = 0.66$ (low SES Ladinos). All correlations are significant, apart from the one exception mentioned above.

Figure 1 illustrates the relation between skeletal and height SDS, which follows a clear positive linear relationship. Yellow dots indicate dental SDS equal or above -2, purple dots indicate dental SDS below -2. The purple dots are almost equally scattered and highlight the lack of association between dental SDS and height SDS or skeletal SDS.

Part 2: The effect of socioeconomic status (SES) and ethnicity (Ladino, Maya) on dental age, skeletal age and height

Social strata and ethnicities (Table 1) differ in height SDS, skeletal age SDS and dental ages SDS (Figure 2). Height SDS differs between all schools (pairwise comparison, $p < .001$, Table 4). Well-nourished middle SES Ladino children were significantly advanced in height, in skeletal age, and in dental age. However, Figure 2 illustrates that the magnitude of this advancement differed between the three variables. Whereas the middle SES children were taller and appeared "older" in skeletal age, the advancement in dental age was small (see also Table 3 for the mean and standard deviation per measurement and cohort). Yet, the leptokurtic distribution of dental SDS reached significance and indicates that the periodically malnourished cohorts of low SES Mayas and Ladino children advanced in dental maturation at slower pace than the well-nourished low-middle and middle class Ladino children.

Discussion

Low SES Mayas are short. Their shortness is associated with very poor social conditions (Bogin and MacVean, 1984) and closely corresponds with a delay in skeletal

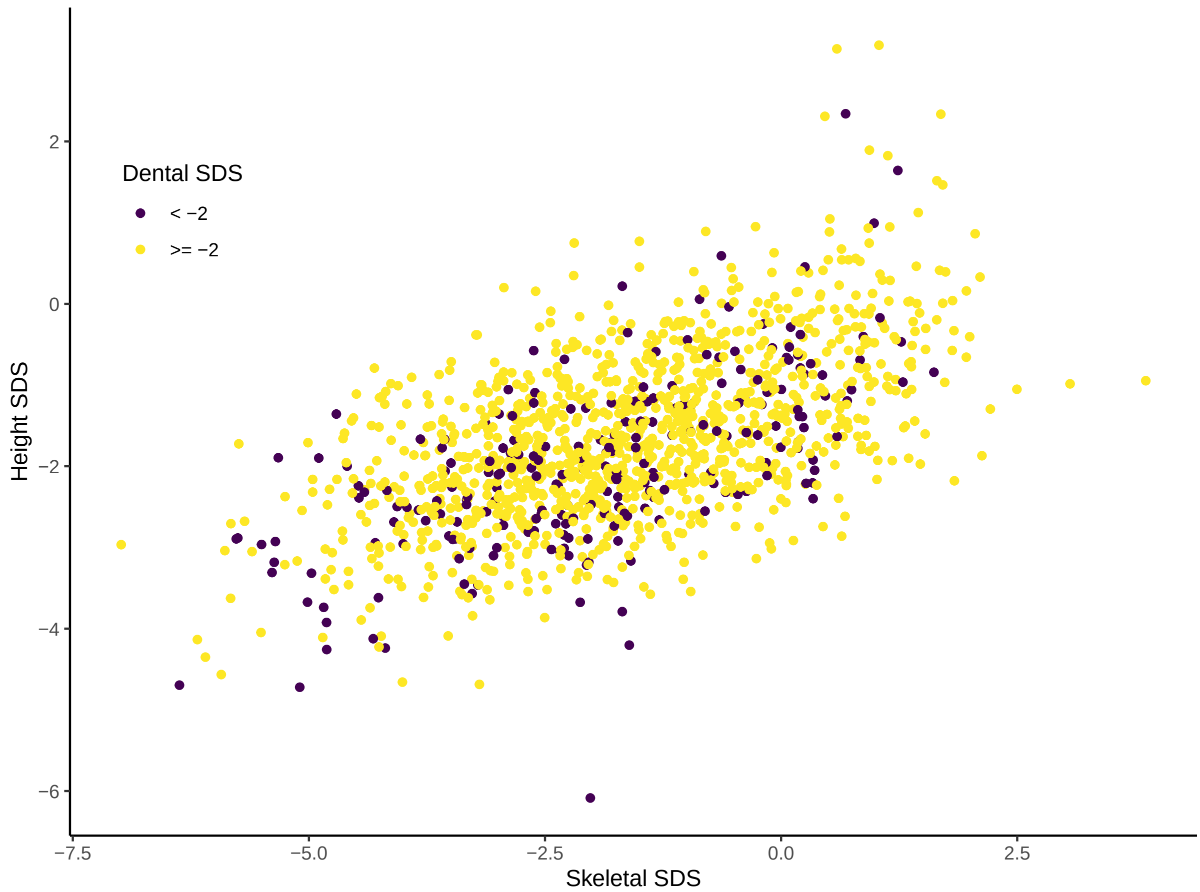

Figure 1 Scatter Plot of skeletal SDS against height SDS of all Guatemalan boys. Red dots indicate individuals that are delayed in dental eruption (dental SDS below -2).

age SDS. However, the correlations between dental maturation and height and dental maturation and skeletal age are low and explain less than 10 % of the variance (3 % and 7 % respectively).

We reject both hypothesis 1, that "dental SDS shows a strong positive correlation with skeletal SDS", and hypothesis 2, that "dental SDS shows a strong positive correlation with height SDS".

The results suggest that the progress in dental age is independent of skeletal age and is instead regulated by different mechanisms. While this is supported by findings in other human populations (Bielicki et al., 1984; Demirjian et al., 1985), these reports are not univocal. Several studies appear to show a dependency between bone age and tooth development. This is difficult to explain and may be due to the lack of standardized methods for analyzing age-dependent variables (Lewis 1991).

Growth depends on bone formation (Beunen et al., 2006; Demirjian et al., 1985). Growth in height largely depends on the formation of longitudinal bones, and thus, on epiphyseal growth that is similar in femur and tibia and in the phalanges. Growth of the mid-face and the teeth differs and appears less sensitive than skeletal growth to environmental influences, such as socioeconomic strata (SES) and ethnicities.

Yet, we find differences between those, had to pay a school fee (midLad, lowmidLad) and those who did not (lowLad, LowMaya), the former showing a small but significant advancement. Previous studies on the same Guatemalan dataset state that low SES Ladino children and especially

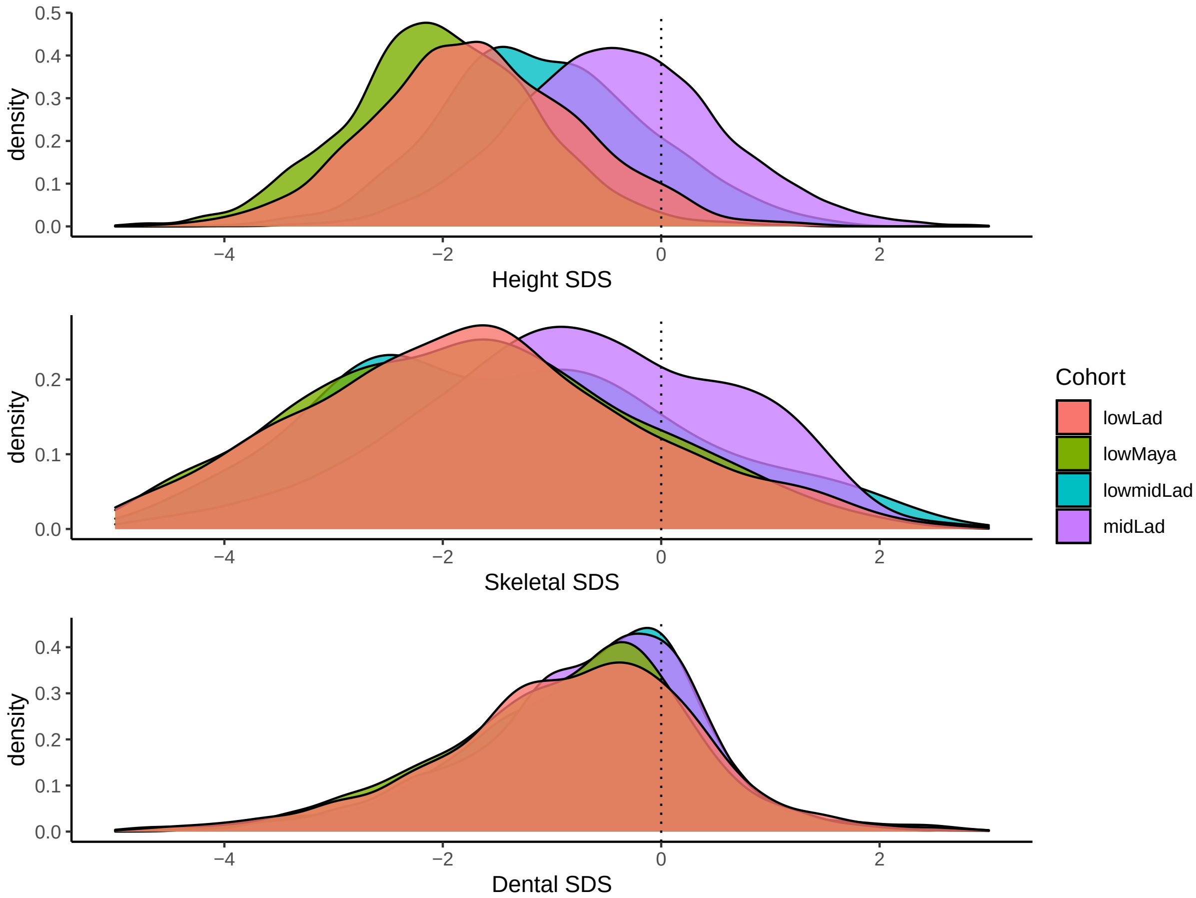

Figure 2 Distribution of SDS for each measurement (dental-, height-, skeletal) per cohort (midLad = middle SES Ladinos, lowmidLad = low-middle SES Ladinos, lowLad = low SES Ladinos, lowMaya = low SES Maya), Within all measurements, there are significant differences between cohorts. Kruskal Wallis Test results: Height SDS: $H(3) = 6050.6$, $p < .001$, skeletal SDS: $H(3) = 480.02$, $p < .001$, dental SDS: $H(3) = 79.586$, $p < .001$.

low SES Maya children suffered from periods of malnutrition (Bogin and MacVean, 1981). While we did not reassess nutritional status in the presented study, we suspect that malnutrition might be the driver for the delay in dental eruption in those two cohorts. This would align with previous findings that identified nutritional status as the main effector of dental development, while other environmental influences, such as social status, show no significant impact (Alhamda, 2012; Demirjian, 1986; Psoter et al., 2008).

There is one major misconception in analyzing the reliability of biological age markers, that we also failed to realize. Namely the notion that developmental markers must produce results similar to skeletal age in order to be considered a good indicator for biological age. We expected such a signal while formulating hypothesis 4: “Cohorts that differ significantly in skeletal age, also differ significantly in dental age”, which we had to reject.

Different organ systems develop at different rates (Beunen et al., 2006). There is not one single “biological age” that can be identified by x-rays of the hand and wrist. “Skeletal age” identified in long bones is not congruent with the state of maturity in dentition. Instead, the present study suggests that even within the skeletal apparatus more than one “biological age” exists. We question that bone age can serve as a “gold standard” of biological age.

Table 3 Mean and standard deviation for height, skeletal and dental SDS per cohort (midLad = middle SES Ladinos, lowmidLad = low-middle SES Ladinos, lowLad = low SES Ladinos, lowMaya = low SES Maya).

SDS	Cohort	mean	sd	Kurtosis	skewness	N
Height	midLad	-0,41	0,97	0,36	0,14	6529
	lowmidLad	-1,06	0,96	1,43	0,42	736
	lowLad	-1,68	0,96	0,31	0,13	3653
	lowMaya	-2,01	0,89	1,24	0,15	4587
Skeletal age	midLad	-0,48	1,32	0,22	-0,09	2540
	lowmidLad	-0,82	1,7	3,38	1,22	90
	lowLad	-1,31	1,45	0,89	0,28	1520
	lowMaya	-1,34	1,42	0,17	0,11	1219
Dental age	midLad	-0,68	1,16	4,66	0,16	4175
	lowmidLad	-0,64	1,22	7,16	0,68	716
	lowLad	-0,85	1,27	2,40	-0,40	2285
	lowMaya	-0,89	1,21	2,49	-0,51	4302

Conclusion

Dental eruption is an independent biological maturation system that is regulated by other mechanisms than skeletal age and height. Dental eruption seems to be is sensitive to malnutrition and may serve as an additional tool to differentiate between malnutrition and other reasons for impaired growth in children, whereas skeletal age is more sensitive to socioeconomic background. In future studies the relationship between nutritional status and dental eruption should be further analyzed.

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Table 4 Summary table of adjusted p-values from the pairwise comparison between cohorts for height-, skeletal-, and dental SDS (midLad = middle SES Ladinos, lowmidLad = low-middle SES Ladinos, lowLad = low SES Ladinos, lowMaya = low SES Maya).

School Pairs	Height SDS		Skeletal SDS		Dental SDS	
midLad x lowLad	p < 0,001	***	p < 0,001	***	p < 0,001	***
midLad x lowmidLad	p < 0,001	***	p = 0,01	*	p = 1,00	
lowLad x lowmidLad	p < 0,001	***	p = 0,08		p = 0,002	**
midLad x lowMaya	p < 0,001	***	p < 0,001	***	p < 0,001	***
lowLad x lowMaya	p < 0,001	***	p = 1,00		p = 1,00	
lowmidLad x lowMaya	p < 0,001	***	p = 0,06		p < 0,001	***

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