Mestrado Integrado em Medicina Dentária

Determination of skeletal maturation in Portuguese children using TW3 Method

Determinação da maturação esquelética em crianças Portuguesas através do Método TW3

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Abstract

Introduction: Determination of skeletal maturity is one of the most reliable parameters to assess a child's growth. This procedure is quite important since skeletal maturity has considerable influence in the diagnosis, prognosis and treatment planning of medical disorders. The Tanner and Whitehouse method for bone age appraisal is a well-established clinical method and has been widely used to study population growth.

Purpose: The aims of this study were: to compare chronological age with bone age; to establish the radio-ulna-short bones (RUS) skeletal maturity of a Portuguese sample; and to compare the skeletal maturation characteristics of a Portuguese sample to others around the world.

Materials and methods: Hand-wrist radiographs of 277 girls and 203 boys, aged 7-16 years old, were rated according to the Tanner-Whitehouse 3 method; smoothed centiles curves of the scores were calculated and compared to those of Belgian, Japanese and Chinese samples.

Results: There were mean differences between skeletal age and chronological age, both for boys and girls. Boys showed significant delay in bone age, with skeletal age lagging behind chronological age, whether the 10-14 year-old girls skeletal age exceeded chronological age. The 50th centiles of the RUS maturity scores showed that Portuguese girls mature faster than the Belgian and the Asian girls, while the Portuguese boys although having a 50^{th} centile RUS scores similar to the Chinese and Japanese, mature faster than the Belgian boys.

Conclusion: Portuguese girls mature faster than the Chinese, Japanese and Belgian girls while Portuguese boys although similar to the Chinese and Japanese mature faster than the other European, the Belgian boys.

Key words: Hand-wrist radiograph, Skeletal maturation, Skeletal age, Bone age, Tanner-Whitehouse method, TW3

Resumo

Introdução: A determinação da maturidade esquelética é um dos parâmetros mais fidedignos utilizado na avaliação do crescimento de uma criança. Este é um procedimento bastante importante, uma vez que a maturidade esquelética de um indivíduo tem uma influência considerável no diagnóstico, prognóstico e plano de tratamento de uma série de distúrbios médicos. O método de Tanner-Whitehouse tem sido amplamente utilizado nos estudos de crescimento populacionais.

Objetivos: Este estudo teve como objectivo comparar a idade cronológica com a idade esquelética, determinar a maturidade esquelética rádio-cúbito-ossos curtos ("RUS") numa amostra portuguesa e comparar as características de maturação esquelética de uma amostra portuguesa com outras internacionais.

Materiais e Métodos: Radiografias da mão e punho de 277 raparigas e 203 rapazes, entre os 7-12 anos de idade, foram analisadas pelo método Tanner-Whitehouse 3. Foram elaborados gráficos dos percentis 50 dos "RUS scores" dos rapazes e raparigas que posteriormente foram comparados com os correspondentes das crianças de outros países.

Resultados: Foram encontradas diferenças significativas entre a idade esquelética e a idade cronológica tanto para os rapazes como para as raparigas. Os rapazes apresentaram, no geral, um atraso na idade esquelética em relação à idade cronológica, enquanto que as raparigas entre os 10-14 anos apresentaram um avanço na idade esquelética em relação à idade cronológica, período sobreponível ao surto pubertário. Os percentis 50 para os "RUS scores" mostraram que as raparigas portuguesas maturam mais cedo do que as belgas e as asiáticas, enquanto que os rapazes portugueses, apesar de terem um percentil 50 sobreponível ao dos rapazes chineses e japoneses, maturam mais cedo do que os belgas.

Conclusões: As raparigas portuguesas maturam mais cedo do que as chinesas, japonesas e belgas; enquanto que os rapazes portugueses apesar de terem uma maturação esquelética semelhante aos chineses e japoneses, maturam mais cedo do que os outros europeus, os rapazes belgas.

Palavras-chave: Radiografia da mão e punho, Maturação esquelética, Idade esquelética, Idade óssea, Método de Tanner-Whitehouse, TW3

Introduction

Growing individuals differ not only on the timing of the maturational events, but also in the sequence of these events (1). Every child has a unique pattern of development and this can have considerable influence on the diagnosis, prognostic, and patients treatment planning (2).

The developmental status of a child can be assessed through various parameters such as height, weight, chronological age, sexual maturation characteristics, skeletal development, dental age (1) and vertebral development (3). Skeletal maturity is a measure of development incorporating the size, shape and degree of mineralization of the bones in order to define the proximity to full maturity (4). Therefore this is a rather important parameter in the diagnosis of endocrine disorders; pediatric syndromes; chronic disease; hormonal therapy; prediction of children height for prognostic and therapeutic purposes (5); forensic science (6–9) and is also rather important in diagnosis and treatment planning in dentofacial orthopedics and orthodontics, especially when growth modification is needed (10).

The classical and most widely used method for skeletal age evaluation is the hand-wrist radiographic analysis (11), mostly because of the wide number of different types of bones available in the area, the low level of radiation needed and the simple radiographic position (12).

Several methods have been developed for the assessment of skeletal age from hand-wrist films, however, none of them are accepted by radiologists as a standard (13). There are two general approaches to assess skeletal maturity by the hand-wrist radiograph (14) namely: the methods described by Björk (15), Grave and Brown (16) or Fishman (17) that identify a limited number of maturation indicators representing ossification events or stages of bone development for each age level (17); the Greulich and Pyle (GP) (18), Tanner and Whitehouse (TW) (19) and FELS methods (20) that create a composite score based on osseous stages and events at each age level.

In 1962, Tanner, Whitehouse and Healy implemented the TW1 method believed to be more flexible and constructed on a more solid mathematical base, than the GP method (19). In 1983, the same authors, using the same standardizing groups of 3000 normal British boys and girls, implemented the TW2 method, a revised version of TW1 (19). In the TW2 the differentiation between genders emerged and some bone stages were eliminated, because they were difficult to rate, and the scores of each level changed. More recently, in 2001, Tanner, Healy, Goldstein and Cameron created the TW3 method (19). This new revision took into account the secular trend in many countries toward a more rapid maturation, and methodological and conceptual advances. Although the description and manual rating of bones stages remained unchanged, i.e., the RUS scores and the Carpal bone were exactly the same in both the TW3 and TW2 methods, the 20-bone score was abolished, the reference values and charts for RUS were changed, taking into account data from North America and from Europe and, consequently, the conversion to obtain bone age also changed (19).

Even though some authors consider that the TW method is more complex, more time-consuming and requires expert knowledge (21), some others ponder the method more flexible and accurate (22).

The TW method has been widely applied in the world (23–38), although few data are available in reference to the skeletal maturation of Portuguese children. Only Freitas *et al.* (35) using the TW2 method in a mixed longitudinal study of youths in Madeira concluded for the RUS and TW2 20-bone scores that the median scores for the boys were advanced, while the girls median scores showed a delay, when compared to those of the Belgian reference. On the other hand the carpal maturity scores showed a delay when compared to those of the Belgian reference.

Therefore the aims of this study were:

1. To compare chronological age with bone age.
2. To establish the RUS skeletal maturity of children in a Portuguese sample.
3. To describe the characteristics of skeletal maturation of a Portuguese sample in comparison to other countries samples.

Materials and methods

Sample

The present study was conducted in the Orthodontic Department consultation at Dental Medicine Area, Faculty of Medicine, University of Coimbra. The sample consisted of 277 Caucasian girls and 203 Caucasian boys raging between 7-15 years of age. None of the individuals involved in the study were reported to have systemic diseases interfering with the growth process or any other disease involving the hands. A good quality left hand-wrist radiograph was obtained for each individual to assess the growth stage and skeletal age.

Assessment of skeletal maturity and observer agreement

All the radiographs were examined with a light box and rated by two independent experts. Skeletal age was estimated using the TW3 method with a computer program (Version 1.0) available in the *TW3 Handbook* (19).

The statistic analyses were performed using the software package SPSS (IBM SPSS Statistics v. 21, Chicago, IL, USA). The 50^{th} centile of RUS scores was calculated and smoothed by means of cubic spline functions; and graphics were obtain with Matlab R2013a.

To assess the internal consistency and interclass correlation, 15 radiographs were randomly selected among all age groups and rated twice by both observers. Without knowledge of each other's ratings and within an 8-day interval, a total of 60 measurements, 30 for each observer were done. Cronback's Alpha and Interclass correlation coefficient (ICC) were then calculated, respectively (Table I and II).

Results

As to the intra-observer assessment, the results were excellent for both observers as Cronbach' Alpha was higher than 0.9 (Table I). The reproducibility of all assessments was good, with a high coefficient value for ICC > 0.98 (*P* > 0.0001), on both readings (Table II).

Table I: Intra-observer assessment (Repeatability or test-retest)

Reliability Statistics	
Observers	Cronbach's Alpha
1st Observer	0.997
2nd Observer	0.998

Table II: Inter-observer assessment (Inter-rater reliability)

Intra-class Correlation Coefficient (ICC)							
	Intra-class Correlation	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
1st reading	.989[a]	0.968	0.996	169.95	14	14	0.000
2nd reading	.995[a]	0.984	0.998	346.554	14	14	0.000

Note: a - the estimator is the same, whether the interaction effect is present or not.

The mean chronological age for the whole sample was 11.92±2.07 years, while the mean bone age for the whole sample was 11.79±2.52 years (Table III). Mean differences between bone age and chronological age were found, both for boys and girls (Table IV and V). For the 10-14 year-old girls, skeletal age exceeded chronological age (Figure 1). Statistical analysis verified that those differences were statistically significant for the 12 year-old girls ($p < 0.0001$) when skeletal maturity is significantly higher than the mean chronological age; and at 15 years old, when mean skeletal age was lower than mean chronological age, signaling full skeletal maturity. For boys there was a trend to bone age to lag behind chronological age, particularly for the 9-13 year-old group. Only the 8 years old boys had a significant skeletal growth.

Table III: Descriptive statistics (size, mean and standard deviation) for chronological age and bone age.

		Total sample	Girls	Boys
Chronological age	N	480	277	203
	Mean	11.92	11.95	11.87
	SD	2.07	2.05	2.10
Bone age	N	480	277	203
	Mean	11.79	12.12	11.35
	SD	2.52	2.47	2.53

Table IV: Comparison of bone and chronological age in girls by age groups.

Age (y)		N	Median	Mean	SD	95% Confidence interval for mean	
						LB	**UB**
7	CA	10	7,58	7,53	0.28	7.33	7.74
	SA	10	8.29	8.91	2.01	6.85	9.72
8	CA	12	8.58	8.63	0.21	8.49	8.76
	BA	12	7.97	7.97	1.19	7.22	8.73
9	CA	30	9.50	9.43	0.26	9.34	9.53
	BA	30	9.24	9.01	1.37	8.50	9.52
10	CA	34	10.54	10.50	0.27	10.41	10.60
	BA	34	10.76	10.84	1.53	10.30	11.37
11	CA	52	11.50	11.48	0.24	11.41	11.54
	BA	52	12.02	11.71	1.27	11.36	12.06
12	CA	47	12.42	12.47	0.26	12.40	12.55
	BA	47	12.68	13.25	1.44	12.83	13.67
13	CA	42	13.42	13.47	0.31	13.37	13.56
	BA	42	13.73	13.66	1.37	13.24	14.09
14	CA	28	14.50	14.48	0.26	14.38	14.58
	BA	28	14.23	14.56	1.01	14.17	14.96
15	CA	22	15.25	15.32	0.38	15.15	15.49
	BA	22	14.48	14.80	1.06	14.33	15.7

Table V: Comparison of bone and chronological age in boys by age groups.

Age (y)		N	Median	Mean	SD	95% Confidence interval for mean	
						LB	UB
7	CA	1	*	-	-	-	-
	BA	1					
8	CA	18	8.42	8.40	0.33	8.24	8.57
	BA	18	8.02	7.90	0.94	7.44	8.37
9	CA	23	9.50	9.42	0.32	9.29	9.56
	BA	23	9.20	9.33	1.19	8.81	9.84
10	CA	28	10.42	10.43	0.25	10.34	10.53
	BA	28	9.86	9.80	1.43	9.24	10.35
11	CA	32	11.54	11.45	0.31	11.34	11.56
	BA	32	10.60	10.49	1.25	10.03	10.93
12	CA	40	12.46	12.47	0.30	12.37	12.56
	BA	40	11.64	11.86	1.66	11.33	12.40
13	CA	23	13.67	13.58	0.30	13.45	13.71
	BA	23	14.05	13.32	1.64	12.62	14.03
14	CA	23	14.42	14.67	0.36	14.31	14.62
	BA	23	14.16	13.95	1.62	13.24	14.65
15	CA	15	15.75	15.50	0.46	15.25	15.75
	BA	15	15.69	15.33	0.81	14.88	15.77

Note: *no results are presented because there was only one boy with 7 years.

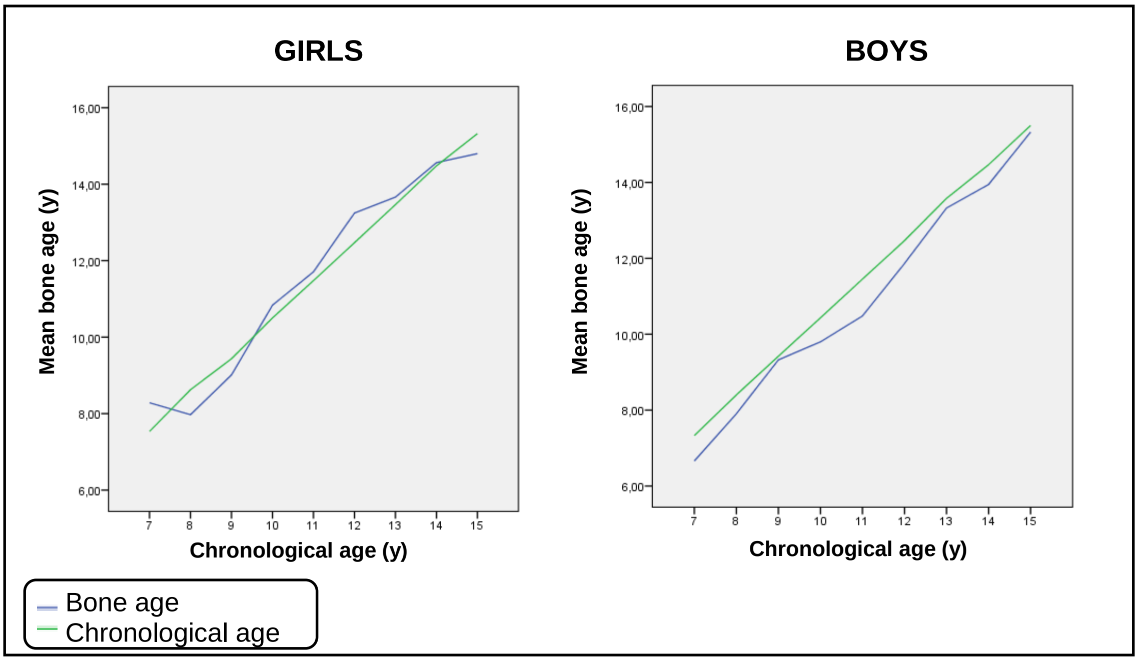

Figure 1: Skeletal maturity chart for girls and boys comparing bone age and chronological age

Table VI and VII illustrates the mean values and/or 50th centiles for chronological age, RUS maturity score and TW3 RUS age, for girls and for boys, respectively.

Table VI: RUS maturity scores, TW3 RUS age (bone age) and chronological age for girls at different age groups

RUS Score – GIRLS											
Age (y)	**N**	**CA**	**TW3**								**CA**
		P50	P5	P10	P25	P50	**RUS Age**	P75	P90	P95	**Mean**
[7-8[	10	7.58	229	235	302	340	8.29	417	470	.	7.53
[8-9[	12	8.58	295	300	336	370	7.97	425	503	.	8.63
[9-10[	30	9.5	311	320	376	444	9.01	488	568	594	9.44
[10-11[	34	10.54	345	425	475	555	10.84	647	715	770	10.50
[11-12[	52	11.5	427	461	569	655	11.71	718	744	752	11.48
[12-13[	47	12.42	560	687	712	743	13.25	866	1000	1000	12.47
[13-14[	42	13.42	500	600	751	831	13.66	871	926	955	13.47
[14-15[	28	14.5	766	848	877	887	14.56	942	1000	1000	14.48
[15-16[	22	15.25	763	859	887	915	14.8	1000	1000	1000	15.32

Table VII: RUS maturity scores, TW3 RUS age (bone age) and chronological age for boys at different age groups.

RUS Score – BOYS											
Age (y)	**N**	**CA**	**TW3**								**CA**
		P50	P5	P10	P25	P50	**RUS Age**	P75	P90	P95	**Mean**
[7-8[	1	7.33	204	204	204	204	6.66	204	204	204	7.33
[8-9[	18	8.42	234	235	241	260	7.91	293	304	.	8.40
[9-10[	23	9.50	242	245	266	287	9.33	325	353	379	9.42
[10-11[	28	10.42	245	250	275	311	9.80	339	389	540	10.43
[11-12[	32	11.54	252	274	230	344	10.48	385	451	490	11.45
[12-13[	40	12.46	294	314	359	403	11.86	526	615	631	12.47
[13-14[	23	13.67	333	358	389	609	13.32	656	741	894	13.58
[14-15[	23	14.42	359	367	546	647	13.95	810	896	985	14.47
[15-16[	15	15.75	606	626	695	842	15.33	851	1000	1000	15.50

The 50th centiles of the RUS maturity scores of Portuguese children in comparison to those from Belgium (30), Japan (32) and China (38) and are shown in Figure 2 (girls) and 3 (boys).

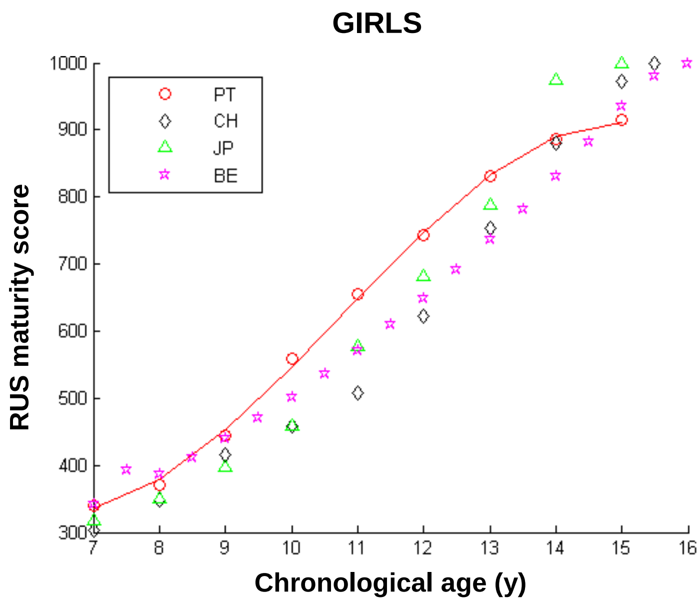

Figure 2: RUS maturity curves (P50) for the Portuguese, Chinese (CH), Japanese (JP) and Belgian (BE) girls

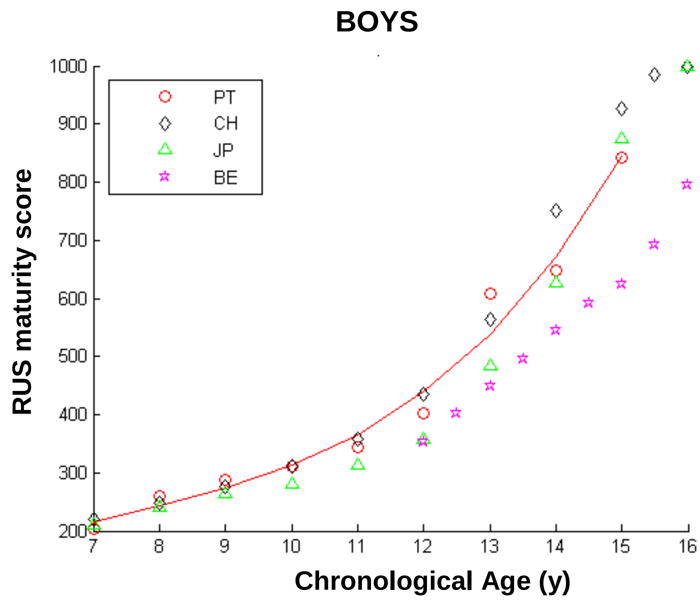

Figure 3: RUS maturity curves (P50) for the Portuguese, Chinese (CH), Japanese (JP) and Belgian (BE) boys

Discussion

Chronological age has been seen as a poor indicator of a child skeletal maturity, thus the hand-wrist radiographs have been widely used to determine bone age, since it is a more reliable and accurate method of assessing individuals' progress toward maturity (39, 40).

The determination of skeletal age by means of hand-wrist radiographs is used by pediatricians, orthodontists, orthopedic surgeons, physical anthropologists and by all of those interested in the study of human growth (41). Growth and physical maturation are dynamic processes encompassing a broad spectrum of cellular and somatic changes. Traditionally, the assessment of growth has placed its primary focus on stature, but changes in body proportions and body composition are essential elements of the growth process. Growth standards have been developed for each of these parameters and aid in the identification of children with normal growth, variations of normal growth and development, as well as in a broad spectrum of abnormal growth states (42). In 2006 the World Health Organization (WHO), released the new WHO Child Growth Standards for preschool children (43) and in 2007 for school-age children and adolescents (44). In Portugal, these curves were implemented in 2013 and are actually present in the Child and Youth Health Book.

In dentofacial orthopedics and orthodontics, in particularly, it is very important to know how the child that is being treated matures, mainly because prognoses can differ according to the onset of treatment.

Regardless the fact that every child has a unique pattern of development it is relevant to construct standards for every population. The standards for estimating skeletal maturity by a given method depend on a specific population rating, that due to ethnic differences, eating habits, socio-economic status and life style translate into different maturation velocities. Therefore, the skeletal age-chronological age equivalents found do not necessarily apply to other populations. Since the TW1 method creation in 1962, many studies have been carried out, all around the world, with a twofold purpose: to construct references values and to quantify possible differences in biological maturation among a given population (35). Even in the same population area because children from the current generation are maturing faster than those from previous ones, the skeletal maturity standards should be revised from time to time. The issue of change, a secular trend in European skeletal maturation, implies the need for further studies (30).

The RUS score is, up to now, one of the best designed, reliable and useful skeletal maturity indices (38). The method is not only 100% rule-based but also experienced-based, using the Golden Series x-rays to train raters (45). Although rater variability is a problem, and greater the experience the lesser the variability, in this particular study, the intra-observer assessment was excellent and the reproducibility of all assessments was good with a high coefficient value for ICC.

In harmony with a previous study (46), in general, the sample skeletal age tended to lag behind chronological age, although both increase in parallel. However for girls between 10-14 years old, the skeletal age exceeded the chronological age, being this period of time related to the pubertal growth spurt. But since a mean and a standard deviation give less meaning information about the sample distribution; the mean values and/or 50th centiles for chronological age, RUS maturity score and TW3 RUS age, for girls and for boys, were obtain. Therefore the 5th (P5), 10th (10), 25th (25), 50Th (P50), 75th (P75), 90th (P90) and 95th (P95) percentiles allowed a better understanding of the skeletal maturity velocity of the sample. The 50th centile was then used to construct a RUS maturity curve (P50) for the Portuguese girls and boys. The smooth curve was than used to compare the Portuguese sample with those of Belgian (BE), Japanese (JP) and Chinese (CH) origin.

Until now the only available Portuguese data came from the Freitas *et al.* (35) mixed longitudinal study done to evaluate the skeletal maturity of youths in Madeira. The study was done according to the TW2 method, assessing the RUS, TW2 20-bone and carpal scores, concluding that for boys the median RUS and the TW2 20-bone scores were higher than those of the Belgian reference, while the median carpal scores were delayed in comparison to the Belgians. As to girls, the median RUS scores were delayed until 11.2 years of age and from this age onward the RUS scores advanced and achieved maturity 1 year earlier than the Belgian girls; median carpal scores showed a delay in all ages and the TW2 20-bone scores were delayed in the 8-12 years old group, after what they showed a slight advancement until full maturity (35). The Belgian reference were more advanced than the British TW standard RUS scores (30).

The present study was done according to the TW3 method; witch considered the carpal scores less reliable, hence the use of only the RUS scores. Nevertheless, when compared to the Belgian sample, the data showed that these Portuguese boys mature faster, with the median RUS scores always higher than those of the Belgian boys, concurring with the Madeira data (Figure 3). As to these Portuguese girls, they showed a delay until the 8 years old, afterwards the median shift and the RUS scores advance in comparison to the Belgians', until the 15 years old when the Belgian girls catch-up (Figure 2). Thus is possible to conclude that during pubertal spurt Portuguese girls mature faster than the Belgian ones. Never forgetting that the Belgian reference was more advanced than the British TW standard RUS scores (30).

As to the Asian samples data comparison, this study median RUS scores for the girls were always advanced until the 14 years old when the Chinese catch-up and Japanese median scores showed a slight advance (Figure 2). The median scores for the study boys overlapped the Chinese and Japanese median RUS scores (Figure 3). The authors of the Japanese study concluded that the Japanese children were advanced in their skeletal maturation during puberty and reach the adult stage earlier than the British TW standards. The RUS maturity of children in Japan progressed rapidly during and after puberty, and they attained the adult RUS maturity stage 1-2 years earlier than Belgian and Chinese (32). The Chinese study concluded that both boys and girls had a slight advancement in RUS skeletal maturation during puberty; but the girls reached full skeletal maturity at the same age as the TW3 children while boys reached full skeletal maturity slightly earlier (38).

Taking into account the results presented in Zhang *et al.* (38), is possible to conclude that the Portuguese girls are advanced especially between 9 and 14 years old, and thenceforth they are delayed, compared with the TW3 girls. Concerning Portuguese boys the only conclusion that can be made is that there are more advanced than TW3 boys until 13 years.

The differences found between populations can be explained by secular trend, genetic factors, by the fact that different observers score the radiographs and maybe because of the lag between the examination years. The different sample sizes also must be considered. Particularly, the study sample doesn’t reach full maturity, implying the need for a larger sample.

Conclusion

Within the scope and limitations of this study is possible to conclude that:

- Portugal needs a Growth Study Center to better understand how the Portuguese population grows, not only for an anthropologic approach, but also for a medical purpose.
- Chronological age is not a reliable parameter to make therapeutic decisions.
- Portuguese's RUS maturity for girls are greater than Belgian, Japanese and Chinese girls between 9 and 13 years.
- Portuguese's RUS maturity for the boys are approximately the same when compared with the other populations.
- More longitudinal studies are needed.

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Bibliography

1. Bala M, Pathak A, Jain RL. Assessment of skeletal age using MP3 and hand-wrist radiographs and its correlation with dental and chronological ages in children. *J Indian Soc Pedod Prevent Dent.* 2010; 28(2):95–9.

2. Turchetta BJ, Fishman LS, Subtelny JD. Facial growth prediction: a comparison of methodologies. *Am J Orthod Dentofacial Orthod.* 2007; 132(4):439–49.

3. San Román P, Palma JC, Oteo MD, Nevado E. Skeletal maturation determined by cervical vertebrae development. *Eur J Orthod.* 2002; 24(3):303–11.

4. Kamal M, Ragini B, Goyal S. Comparative evaluation of hand wrist radiographs with cervical vertebrae for skeletal maturation in 10-12 years old children. *J Indian Soc Pedod Prev.* Dent 2006; 24(3):127-35.

5. Bertaina C, Stasiowska B, Benso a, Vannelli S. Is TW3 height prediction more accurate than TW2? Preliminary data. *Horm Res.* 2007; 67(5):220–3.

6. Santoro V, Roca R, De Donno A, Fiandaca C, Pinto G, Tafuri S, *et al*. Applicability of Greulich and Pyle and Demirijan aging methods to a sample of Italian population. *Forensic Sci Int.* 2012; 221(1-3):153.e1–5.

7. Patil ST, Parchand MP, Meshram MM, Kamdi NY. Applicability of Greulich and Pyle skeletal age standards to Indian children. *Forensic Sci Int.* 2012; 216(1-3):200.e1–4.

8. Santoro V, De Donno A, Marrone M, Campobasso C P, Introna F. Forensic age estimation of living individuals: a retrospective analysis. *Forensic Sci Int.* 2009; 193(1-3):129.e1–4.

9. Santos C, Ferreira M, Alves FC, Cunha E. Comparative study of Greulich and Pyle Atlas and Maturos 4.0 program for age estimation in a Portuguese sample. *Forensic Sci Int.* 2011; 212(1-3):276.e1–7.

10. Soegiharto BM, Cunningham SJ, Moles DR. Skeletal maturation in Indonesian and white children assessed with hand-wrist and cervical vertebrae methods. *Am J Orthod Denfacial Orthop.* 2008; 134(2):217–26.

11. Gandini P, Mancini M, Andreani F. A comparison of hand-wrist bone and cervical vertebral analyses in measuring skeletal maturation. *Angle Orthod.* 2006; 76(6):984–9.

12. Cameriere R, Ferrante L, Mirtella D, Cingolani M. Carpals and epiphyses of radius and ulna as age indicators. *Int J Legal Med.* 2006; 120(3):143–6.

13. Pietka E. Computer-assisted bone age assessment based on features automatically extracted from a hand radiograph. *Comput Med Imaging Graph.* 1995; 19(3):251-9.

14. Flores-Mir C, Nebbe B, Major PW. Use of skeletal maturation based on hand-wrist radiographic analysis as a predictor of facial growth: a systematic review. *Angle Orthod.* 2004; 74(1):118–24.

15. Björk A. Timing of interceptive orthodontic measures based on stages of maturation. *Trans Eur Orthod Soc.* 1972; 61-74.

16. Grave KC, Brown T. Skeletal ossification and the adolescent growth spurt. *Am J Orthod.* 1976; 69(6):611–9.

17. Fishman LS. Radiographic evaluation of skeletal maturation. A clinically oriented method based on hand-wrist films. *Angle Orthod.* 1982; 52(2):88-112.

18. Greulich WW, Pyle SI. Radiographic Atlas of Skeletal Development of Hand and Wrist, 2nd edn. 1959. California: Stanford University Press.

19. Tanner JM, Healy MJR, Goldstein H, Cameron N. Assessment of skeletal maturity and prediction od adult weight (TW3 Method). 2001. Edinburgh: Saunders.

20. Malina RM, Chamorro M, Serratosa L, Morate F. TW3 and Fels skeletal ages in elite youth soccer players. *Ann Hum Biol.* 2007; 34(2):265-72.

21. Sato K, Mito T, Mitani H. An accurate method of predicting mandibular growth potential based on bone maturity. *Am J Orthod Dentofacial Orthod.* 2001; 120(3):286–93.

22. Mentzel HJ, Vilser C, Eulenstein M, Schwartz T, Vogt S, Böttcher J, *et al.* Assessment of skeletal age at the wrist in children with a new ultrasound device. *Pediatr Radiol.* 2005; 35(4):429–33.

23. Kimura K. Skeletal maturity of the hand and wrist in Japanese children by the TW2 method. *Ann Hum Biol.* 1977; 4(4):353–6.

24. Kimura K. Skeletal maturity of the hand and wrist in Japanese children in Sapporo by the TW2 method. *Ann Hum Biol.* 1977; 4(5):449–53.

25. Van Venrooij-Ysselmuiden ME, Van Ipenburg A. Mixed longitudinal data on skeletal age from a group of Dutch children living in Utrecht and surroundings. *Ann Hum Biol.* 1978; 5(4):359–80.

26. Helm S. Skeletal Maturity in Danish schoolchildren assessed by the TW2 method. *Am J Phys Anthropol.* 1979; 51(3):345-52.

27. Wenzel A, Melsen B. Skeletal maturity in 6–16-year-old Danish children assessed by the Tanner-Whitehouse-2 method. *Ann of Hum Biol.* 1982; 9(3):277–81.

28. Wenzel A, Droschl H, Melsen B. Skeletal maturity in Austrian children assessed by the GP and the TW-2 methods. *Ann Hum Biol.* 1984; 11(2):173–7.

29. Zhen O, Baolin L. Skeletal maturity of the hand and wrist in Chinese school children in Harbin assessed by the TW2 method. *Ann Hum Biol.* 1986; 13(2):183–7.

30. Beunen G, Lefevre J, Ostyn M, Renson R, Simons J, Van Gerven D. Skeletal maturity in Belgian youths assessed by the Tanner-Whitehouse method (TW2). *Ann Hum Biol.* 1990; 17(5):355–76.

31. Ye Y, Wang C, Cao L. Skeletal maturity of the hand and wrist in Chinese children in Changsha assessed by TW2 method. *Ann Hum Biol.* 1992; 19(4):427–30.

32. Ashizawa K, Asami T, Anzo M, Matsuo N, Matsuoka H, Murata M, et al. Standard RUS skeletal maturation of Tokyo children. *Ann Hum Biol.* 1996; 23(6):457–69.

33. Lejarraga H, Guimarey L, Orazi V. Skeletal maturity of the hand and wrist of healthy Argentinian children aged 4–12 years, assessed by the TWII method. *Ann Hum Biol.* 1997; 24(3):257–61.

34. Tanner J, Oshman D, Bahhage F, Healy M. Tanner-Whitehouse bone age reference values for North American children. *J Pediatr.* 1997; 131:34-40.

35. Freitas D, Maia J, Beunen G, Lefevre J, Claessens A, Marques A, Rodrigues A, Silva C, Crespo M, Thomis M, Sousa A, Malina R. Skeletal maturity and socio-economic status in Portuguese children and youths: the Madeira growth study. *Ann Hum Biol.* 2004; 31(4):408–20.

36. Molinari L, Gasser T, Largo RH. TW3 bone age: RUS/CB and gender diferences of percentiles for score and score increments. *Ann Hum Biol.* 2004; 31(4):421-35.

37. Ashizawa K, Kumakura C, Zhou X, Jin F, Cao J. RUS skeletal maturity of children in Beijing. *Ann Hum Biol.* 2005; 32(3):316–25.

38. Zhang SY, Liu LJ, Wu ZL, Liu G, Ma ZG, Shen XZ, *et al*. Standards of TW3 skeletal maturity for Chinese children. *Ann Hum biol.* 2008; 35(3):349–54.

39. Caldas Mde P, Ambrosano GM, Haiter Neto F. New formula to objectively evaluate skeletal maturation using cephalometric radiographs. *Braz Oral Res.* 2007; 21(4):330-5.

40. Silveira AM, Fishman LS, Subtelny JD, Kassebaum DK. Facial growth during adolescence in early, average and late matures. *Angle Orthod.* 1992; 62(3):185-90.

41. Gilsanz V, Ratib O. Hand Bone Age A Digital Atlas of Skeletal Maturity, 2nd Edn 2012. Springer.

42. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. *J Adolesc Health.* 2002; 31(6 Suppl):192-200.

43. WHO Multicentre Growth Reference Study Group. WHO Child growth standards Standards based on length/height, weight and age. *Acta Paediatr Suppl.* 2006; 450:76-85.

44. De Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Devolpment of a WHO growth reference for school-aged children and adolescentes. *Bull World Health Organ.* 2007; 85(9):660-7.

45. Thodberg HH. An autometed method for determination of bone age. *J Clin Endocrinol Metab.* 2009; 94:2239-2244.

46. Buckler JM. Skeletal age changes in puberty. *Arch Dis Child.* 1984; 59(2):115–9.