

ORIGINAL ARTICLE

Five radiographic methods for assessing skeletal maturity in a Spanish population: is there a correlation?

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ABSTRACT

Objectives: The need for accurate techniques of estimating age has sharply increased in line with the rise in illegal migration and the political, economic and socio-demographic problems that this poses in developed countries today. The methods routinely employed for determining chronological age are mainly based on determining skeletal maturation using radiological techniques. The objective of this study was to correlate five different methods for assessing skeletal maturation.

Materials and methods: 606 radiographs of growing patients were analyzed, and each patient was classified according to two cervical vertebral-based methods, two hand-wrist-based methods and one tooth-based method. Spearman's rank-order correlation coefficient was applied to assess the relationship between chronological age and the five methods of assessing maturation, as well as correlations between the five methods ($p < 0.05$).

Results: Spearman's rank correlation coefficients for chronological age and cervical vertebral maturation stage using both methods were 0.656/0.693 ($p < 0.001$), respectively, for males. For females, the correlation was stronger for both methods. The correlation coefficients for chronological age against the two hand-wrist assessment methods were statistically significant only for Fishman's method, 0.722 ($p < 0.001$) and 0.839 ($p < 0.001$), respectively for males and females.

Conclusions: The cervical vertebral, hand-wrist and dental maturation methods of assessment were all found to correlate strongly with each other, irrespective of gender, except for Grave and Brown's method. The results found the strongest correlation between the second molars and females, and the second premolar and males.

Clinical relevance: This study sheds light on and correlates with the five radiographic methods most commonly used for assessing skeletal maturation in a Spanish population in southern Europe.

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Introduction

Age assessment is an important aspect of medicolegal, paediatric and forensic science. Estimating the age of a living individual who cannot prove his/her birth date poses a challenge, which results in a fairly common legal practice. The need for accurate techniques of estimating skeletal maturation has sharply increased in line with the increase in illegal migration, which is a growing political, economical and socio-demographical problem in developed countries today. In cases involving criminal proceedings, the evaluation of imputability or asylum is essential to be able to find out the age of someone who has no identification documents, since the chronological age limits defining full or legal responsibility vary considerably in different countries around the world.[1,2] In most European countries, the legal age limit falls between the 14 and 21 years of life; in the case of Spain, the age of criminal responsibility is 14 years.[3]

The methods routinely used to determine chronological age are mainly based on determining biological age.[2,4–7] Various indicators of maturity such as sexual maturation

characters,[8–10] dental development,[5,11–13] hand-wrist maturity [14,15] and cervical vertebral maturation [16–18] have been reported in the specialized literature for estimating the chronological age of children and adolescents. Voice change needs to be continuously monitored in order to detect changes, while the menarche has little predictive value if it has not occurred or if the patient cannot remember exactly when the first menstrual period was. To date, therefore, the use of radiographic growth parameters has been the most commonly used method of assessing biological age and so determining chronological age. Hand-wrist radiography has been one of the most commonly used methods of determining skeletal maturity, although cervical vertebral maturation and dental development have been proposed as valid alternatives to the hand-wrist method. Many authors have reported a significant correlation between the size and shape of the cervical vertebrae, weight and body height [19] and hand-wrist maturation.[17,20,21]

In Greulich and Pyle's [6] and Tanner et al.'s [22] methods, bone age is assessed by comparing the X-ray image of an unknown subject with a standard reference.[23] Among the

methods used to identify the events of ossification, the most frequently used are Fishman's method [24] and Grave and Brown's method,[15] which are mainly used for orthodontics diagnosis but have been applied for forensic purposes. The first involves 11 skeletal maturity indicators (SMIs) in six anatomical regions, and the second evaluates 14 ossification events. Hassel and Farman [17] established a significant relationship between Fishman's method of using hand-wrist radiography to assess skeletal maturity and assessing maturity with lateral cephalometric radiography taking only the second, third and fourth cervical vertebrae, unlike Lamparski's method, which used C2-C6.[16] Another simplified method, Baccetti's method,[25] assesses the shape of the inferior border of C2-C3 and C4 and their body structure. Dental age is one of the many physiological characteristics that have been established as individual diagnostic methods.[26–28] Many authors have highlighted the association between dental age (eruption and/or calcification) and skeletal age.[29–33] In this respect, there is considerable controversy in the literature,[34–36] although numerous studies agree that there is substantial inter-population variation within this association.[13] The research objective therefore was to evaluate the relationships between five different methods described in the literature for assessing skeletal maturation in a selected population. More specifically, the objective of the present study was to associate chronological age, stage of tooth calcification, skeletal maturation of the hand and wrist and cervical vertebral maturation on lateral, hand-wrist and panoramic radiographs, in order to determine whether or not these methods are valid clinical tools for assessing skeletal maturation and the degree of correlation between them.

Materials and methods

Ethics statement

This research follows ethical guidelines governing medical research, as described in the Helsinki Declaration (www.wma.net/e/policy/b3.htm/2002 version), along with approval for experimentation by the designated Institutional Ethical Committee of the US.

Population

From 6200 radiographs (taken from a total of 2068 patients), 606 radiographs (lateral, panoramic and hand-wrist radiographs of each patient) of 202 growing subjects (104 boys and 98 girls) were finally selected from the Department of Orthodontics in the University of Seville, Spain, who provided informed consent. The selected subjects were Spanish Caucasian, of middle-class socio-economic status, and their mean age was 12 years and 9 months [SD: ± 1 year and 8 months] (13 years and 4 months for males [SD: ± 1 year and 7 months], 12 years and 7 months for females [SD: ± 1 year and 9 months]). The criteria for inclusion for the selected patients were as follows: (1) Spanish subject (three generations of Spanish ancestry); (2) subjects with normal systemic growth and no serious illnesses; (3) no previous tooth

trauma; (4) no agenesis, root canal treatment or alteration of evaluated teeth; (5) no history of injury or malformation to the craniofacial or hand-wrist region; (6) no previous orthodontic treatment; and (7) bones and teeth showed up clearly on radiographs. Subjects with one or more poorly recorded radiographs were excluded from the study, as were those wearing a lingual arch.

Radiographic records and skeletal maturation assessment

Lateral, hand-wrist and panoramic radiographs were taken of each subject on the same visit. The same machine was used for all radiographic projections.

Radiographic cervical vertebrae assessment

Cervical vertebral maturation stage (CVMS) was evaluated using the two well-described methods of Hassel and Farman [17] and Baccetti et al.[18] Briefly, CVMS assessment with Hassel and Farman's method involved evaluating morphological modifications to the second to the fourth cervical vertebrae with six categories of cervical vertebral skeletal maturation: initiation (CVM1); acceleration (CVM2); transition (CVM3); deceleration (CVM4); maturation (CVM5); completion (CVM6).[17]

Baccetti et al.'s adaptation of this method of vertebral assessment [18] fuses Hassel and Farman's CVM1 and CVM2 stages into one and defines them as follows: cervical stage 1 (CS1); cervical stage 2 (CS2); cervical stage 3 (CS3); cervical stage 4(CS4); cervical stage 5(CS5); and cervical stage 6(CS6).[18]

Radiographic hand-wrist assessment

Two methods were used to evaluate skeletal maturity from hand-wrist radiographs: Björk's method,[37] as modified by Grave and Brown,[15] and referred to henceforth as Grave and Brown's method. Briefly, 14 ossification events on the pisiform, radius, hamate and the first, second and third fingers were evaluated, which were then grouped into 9 stages of maturation.[21,38,39] The second method was Fishman's.[8] In summary, this method involves 11 SMIs in six regions situated on the thumb, radius, and third and fifth fingers. Individual maturity indicators were classified according to 11 stages of maturity.

Radiographic dental age assessment

Skeletal maturity of different stages of tooth calcification was evaluated on panoramic radiographs on the first and second premolars and the first and second molars, following Demirjian's measurement technique.[5] Dental stage was calculated using mandibular teeth on the left side; no maxillary teeth were used since superimposed anatomical structures in this area (overlap of palate, zygomatic arch, maxillary sinus, root) make it difficult to assess the calcification stage of the teeth. This method uses eight stages of calcification, from A to H, in its classification.[5]

Statistics

To calculate intra/interexaminer reproducibility of the ratings of each of the five methods, the Spearman–Brown formula was used. The hand-wrist, panoramic and lateral radiographic records of 15 randomly selected patients were examined and evaluated by the same experienced operators five-weeks after the initial rating. Another three experienced examiners tested interexaminer reliability, by analyzing 15 randomly chosen radiographic records.

To quantify the association between the different methods, Spearman's coefficient of correlation was applied to calculate the association between chronological age and the five methods of assessing maturation, as well as the correlations between the five methods. Significance level was set at a p value of 0.05. All statistical analyses were performed with SPSS software version 170.0 (SPSS Inc., Chicago, IL).

Results

The reproducibility of all evaluations was reliable, with good concordance coefficients. The reliability coefficients ranged between 0.832 and 0.961 for the cervical vertebral evaluations, between 0.801 and 0.941 for the hand-wrist assessments, and between 0.79 and 0.898 for the dental assessments, for both inter-examiner and intra-examiner reliability.

Frequencies of cervical vertebrae stage using Hassel and Farman's and Baccetti et al.'s methods are shown in Table 1. Spearman's rank correlation coefficients for chronological age and CVMS for males, using Hassel and Farman and Baccetti et al., were 0.656 and 0.693 ($p < 0.001$), respectively. For females, the correlation was stronger for both methods, at 0.713 and 0.747 ($p < 0.001$), respectively (Table 2).

The most frequently occurring stages of hand-wrist skeletal maturation using Fishman's method were stage 11 (21.4%) in females, and stage 3 (25.5%) for males (Table 1). Using Grave and Brown's method, the most frequent stages of hand-wrist maturation in females were, in descending order: stage 5 (24.0%), 3 (24.0%), 9 (16.7%) and similarly for

males: 5 (31.1%), 3(24.3%), 9 (23.3%) (Table 1). The correlation coefficients for chronological age against the two hand-wrist assessment methods of Fishman and Grave and Brown were 0.722 ($p < 0.001$) and 0.82 ($p = 0.41$), respectively, for males, and 0.839 ($p < 0.001$) and 0.54 ($p = 0.6$), respectively, for females (Table 2). The association between Fishman's assessment and chronological age ($p < 0.001$) was stronger in females than in males. No statistically significant association ($p > 0.05$) between age and Grave and Brown's method was found however for either gender (Table 2).

For evaluating dental age using Demirjian, the most frequently observed stages for first premolars in males were stage H (45.2%) and G (31.7%), and in females, stage H (46.9%) and G (30.6%). For second premolars, the results for males were: stage G (36.5%) and F (34.6%), and for females: stage F (41.8%) and G (28.6%). For the first molar in males and females, the results were: stages H (84.6% and 85.7%) and G (7.7% and 8.2%), respectively, and for the second molar in males and females: stages G (39.4% and 34.7%) and F (26.9% and 31.6%), respectively (Table 1). For the association between chronological age and dental maturation according to Demirjian, the coefficients for those teeth measured for males and females, respectively, were: first molar 0.378 ($p < 0.001$) and 0.311 ($p = 0.002$); second molar 0.748 and 0.767 ($p < 0.001$); first premolar 0.636 and 0.675 ($p < 0.001$); second premolar 0.634 and 0.462 ($p < 0.001$). Statistically significant associations were found for males and females, with a strong correlation for all teeth in males, and with all teeth except for the first molar in females, which correlated less strongly (Table 2).

The association between the hand-wrist and cervical vertebral maturation methods is shown in Table 3. In summary, the two cervical vertebrae methods obtained coefficients of 0.826 ($p < 0.001$) for Hassel and Farman's method against Fishman's method, and 0.831 ($p < 0.001$) for Baccetti et al.'s method against Fishman's method. Whereas a statistically significant association was observed for the Fishman method, there was none between Grave and Brown's method and either of the two cervical vertebral assessments ($p > 0.05$) (Table 3).

Table 1. Sample distribution according to cervical vertebrae stages (Hassel and Farman's and Baccetti et al.'s methods), hand-wrist stages (Fishman and Grave and Brown's methods) and dental calcification stages (Demirjian's method).

Maturation/ Calcification stages	Cervical vertebrae M				Hand-wrist M				Dental M							
	H&F's (m/f)		B's (m/f)		Fishman's (m/f)		G&B's (m/f)		1PM		2PM		1M		2M	
Stg 1/Stg A	25	15	7	5	6	2	1	1			3	6	0	0	0	0
Stg 2/Stg B	25	14	21	13	8	2	1	1			0	0	0	0	0	0
Stg 3/Stg C	28	20	31	17	26	10	25	23			1	0	0	0	0	1
Stg 4/Stg D	10	13	26	19	18	14	5	12	2	0	1	1	0	0	5	3
Stg 5/Stg E	7	27	10	34	7	5	32	23	0	2	5	5	0	0	21	16
Stg 6/Stg F	9	9	9	10	6	6	6	6	22	20	30	39	1	1	28	31
Stg 7/Stg G					11	8	5	9	33	30	37	28	8	8	41	34
Stg 8/Stg H					2	11	4	5	47	46	20	17	88	84	8	11
Stg 9					5	9	24	16								
Stg 10					5	10										
Stg 11					8	21										
TOTAL	202		202		200		199		202		193		190		199	

Stg: stage; H&F's: Hassel and Farman's method; B's: Baccetti et al.'s method; G&B's: Grave and Brown's method; Dmjn's: Demirjian's method; (m/f): males sample/females sample; 1PM: first premolar; 2PM: second premolar; 1M: first molar; 2M: second molar; M: methods.

Table 2. Distribution and correlation coefficients between cervical maturation, hand-wrist maturation or dental calcification stages and chronological age of subjects.

Chronological age (years old)	Cervical vertebrae CC					Hand-wrist CC					Dental CC											
	H&F's (m/f)		B's (m/f)		r(m/f)	Fishman's (m/f)		G&B's (m/f)		r(m/f)	1PM		2PM		1M		2M					
	5-9	9-12	12-15	15-18	TOTAL	2/3	24/38	63/45	13/12	200	2/3	24/37	64/44	13/12	199	2/3	22/36	61/43	12/11	190		
	5-9	9-12	12-15	15-18	TOTAL	2/3	24/38	63/45	13/12	200	2/3	24/37	64/44	13/12	199	2/3	22/36	61/43	12/11	190		
						r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	r(m/f)	
						.656**/.713**	.693**/.747**	.722**/.839**	.82/.54	.636**/.675**	.634**/.462**	.378**/.311*	.748**/.767**									

CC: correlation coefficients with chronological age of subjects; H&F's (m/f): Hassel and Farman's method frequencies for males and females; B's (m/f): Baccetti et al.'s method frequencies for males and females; G&B: Grave and Brown's method frequencies for males and females; Dmjn's: Dermijans method frequencies for males and females; r (m/f): r significance for males/r significance for females (Spearman's rho coefficient analysis); 1PM: first premolar; 2PM: second premolar; 1M: first molar; 2M: second molar.
 * $p < 0.05$.
 ** $p < 0.001$.

Table 3 shows Spearman's correlation coefficients for cervical vertebral maturation against dental maturation. The coefficients ranged between 0.620 ($p < 0.001$) and 0.257 ($p < 0.05$) in males, and between 0.611 ($p < 0.001$) and 0.167 ($p > 0.05$) in females. The level of significance was similar for all coefficients ($p < 0.001$ or $p < 0.05$), except for the first molar maturation assessment in girls, which was not statistically significant. For male patients, the sequence, from lowest to highest, against the two methods of cervical vertebral maturation was: first molar, first premolar, second molar and premolar. For female patients, the sequence, from lowest to highest, was: first molar, second bicuspid, first premolar and second molar (Table 3).

The results of the correlation between hand-wrist assessment methods and dental maturation are compiled in Table 3. There was no statistical association, for girls or boys, between Grave and Brown's method and dental maturation ($p > 0.05$); Fishman's method was significantly associated with all stages of maturation in boys and girls, with the exception of the first molar in boys ($p = 0.6$).

In addition, the two cervical vertebrae maturation methods correlated well in boys (0.949; $p < 0.001$) and girls (0.963; $p < 0.001$). Nonetheless, the two hand-wrist evaluation methods showed no association, irrespective of gender ($p > 0.05$) (Table 3).

Discussion

The association between different methods of assessing skeletal and dental maturation has been studied in various populations. Several authors have suggested that ethnic origin and region-dependent factors such as climate, among others, may play some part in influencing associations in methods of assessing maturation used in dentofacial orthopedics.[13,38] Based on this assumption, our aim was to determine whether there were correlations between the five different methods of assessing maturation in a southern European (Spanish) population.

Although the hand-wrist and cervical vertebral maturation methods of assessing biological age are mainly used in the field of orthodontics, they have also been used to determine chronological age for forensic purposes. We examined two well-known methods for evaluating hand-wrist maturation, two well-described methods of assessing cervical vertebral maturation and finally, the stage of dental maturation. Many authors have supported the efficacy of cervical vertebral analysis, with various available methods of assessing skeletal age.[17,18] Baccetti's and Hassel and Farman's methods were chosen because they use the number of vertebrae that can be evaluated with a protective collar.[16-18,39]

Many other authors have found statistically significant correlations in various populations between methods of assessing skeletal maturation in the hand-wrist and cervical vertebrae.[16,17,38,39] As was the case in those publications, our results in a Spanish population showed a strong statistical correlation between hand-wrist maturation (Fishman) and the two approaches for evaluating the shape of cervical vertebrae (Baccetti et al. and Hassel & Farman), irrespective

Table 3. Correlation coefficients between cervical maturation, hand-wrist maturation and dental maturation stages of subjects.

		Tooth	Cervical vertebrae CC		Hand-wrist CC	
			H&F's (m/f)	B's (m/f)	Fishman's (m/f)	G&B's (m/f)
Dental methods	Demirijyan's method	1PM	.519**/.560**	.532**/.561**	.482**/.672**	.010/-.053
		2PM	.613**/.386**	.620**/.405**	.594**/.465**	.040/-.002
		1M	.613**/.167	.257*/.176	.187/.263*	-.045/-.069
		2M	.613**/.582**	.584**/.611**	.569**/.711**	.111/-.055
Cervical Vertebrae methods	H&F's	-	-	0.826**	0.03	
	B's (m/f)	-	-	0.831**	0.12	

CC: correlation coefficients with dental maturation stages according to Demirijyan's method; H&F's: Hassel and Farman's method; B's: Baccetti et al.'s method; (m/f): *r* significance for males/*r* significance for females (Spearman's rho coefficient analysis); Fishman's: Fishman's method G&B's: Grave and Brown's method; 1PM: first mandibular premolar; 2PM: second mandibular premolar; 1M: first mandibular molar; 2M: second mandibular molar.

* $p < 0.05$.

** $p < 0.001$.

of gender. However, we found none in our population between Grave and Brown's method and either of the cervical vertebral maturation methods.

Several researchers have found a relatively high correlation between chronological age and skeletal maturation, with coefficients ranging from 0.58 to 0.71 for Caucasian children in an American population,[40] and from 0.72 for cervical vertebral assessment to 0.79 using hand-wrist assessment, in a Turkish population.[38] Similarly, in a southern Chinese population,[41] there were correlations between cervical vertebral maturity and chronological age (0.74 for males; 0.787 for females) and between hand-wrist maturity and chronological age (0.7492 for males; 0.7758 for females). Other authors found no correlation between the hand-wrist method and chronological age.[42] In accordance with previous findings in the populations mentioned, our study found that chronological age correlated with a hand-wrist assessment (Fishman's method, but not Grave & Brown's), cervical vertebral maturation (Hassel & Farman and Baccetti et al.) and with dental maturation (Demirjian method) in a Spanish population.

With respect to gender, many authors have suggested that the association between vertebral stage of development and hand-wrist development is more reliable in women than in men.[16,38,39] Although we found a strong correlation in both females and males, females showed a slightly stronger association using these two methods. Gender differences in our population were found for the association between the cervical vertebral assessment methods and the calcification stage of the first molar according to Demirjian, which did not correlate in females but did in males ($p < 0.05$). Conversely, Fishman's hand-wrist assessment did not correlate with tooth calcification in males, but did in females ($r = 0.263$; $p = 0.009$).

To determine dental age, several methods can be used.[5,43,44] The most relevant in the literature are fundamentally concerned with those based on time of eruption,[45] calcification or root formation.[46] The disadvantages of methods based on time of eruption are that they depend on local factors and systemic diseases (environmental influence) [47] and that it is very difficult to determine the exact time of eruption. In our study, we took Demirjian's method, which is based on using measurements from radiographs with objective shape criteria and proportions of root length versus crown height (not absolute length).[5,48] The mandibular first/second premolars and first/second molars were considered in

this study. Previous studies used either mandibular canines [49] or third molar examination,[50] although we declined to use these teeth since they did not come out clearly on radiographs, showed wide variability and were also commonly missing teeth.

The lack of unanimous agreement in the literature about the association between teeth calcification stage and SMIs could be due, at least in part, to the different approaches used to determine skeletal and dental stage, as well as to population differences. Some authors have found a strong association [13,40,43] between them and others a low one.[51,52] The results of this study found the strongest correlation between second molars and females, which coincide with other studies.[53–55] Nevertheless, differences were found for males, with the strongest correlation found being with the second mandibular premolar. Differences in data compared with other studies may be due to differences in ethnicity or methodology.

It is essential to find an efficient, accurate and reliable tool for estimating the age of living people because of the legal consequences, such as penal prosecution, criminal responsibility and refusal of asylum, facing migrants without valid identity papers. Because of the increase in illegal immigration, particularly in the Mediterranean area (in other words, Italy, Malta, Greece and Spain), estimating age has become an important issue in forensic anthropology.[4] Contemporary forensic reports have suggested a multidisciplinary approach that takes into account many different methods, like physical examination, tooth and skeletal development, in order to obtain more reliable age assessments.[2,53] As limitations of the present study, it should be borne in mind that the results obtained in this study may be valid only for the Spanish population due to the ethnic variation mentioned above and it is recommended that the sample be extended due to the variation in age distributions and the different stages of maturation. As future objectives, the assessment of other methods, such as those assessing the middle phalanx of the third finger (MP3 method) [56] or Greulich and Pyle's method, may be of interest for the line of research followed in the present study.[6]

Conclusions

The cervical vertebrae (Hassel & Farman or Baccetti et al.), hand-wrist (Fishman) and dental maturation (Demirjian)

methods of assessment were all found to correlate strongly with each other, irrespective of gender, in the subjects of southern European (Spanish origin) used in this study. There was lack of correlation only between Fishman's method and first molar stage in males, and between the first molar and the two methods of assessing cervical vertebrae examined (Hassel & Farman and Baccetti et al.) in females. Chronological age showed good correlation with all methods, except Grave and Brown's. This correlation is stronger in females, except for the first molar in the dental method. The mandibular second molar and the mandibular second premolar offered the most reliable maturation index for females and males respectively, which makes the panoramic radiograph an easy, useful and valid tool for identifying skeletal and chronological age. Furthermore, while there was a strong correlation between Fishman's method for hand-wrist assessment and the vertebral assessment methods, there was none between Grave and Brown's and any of the other four methods used in the population of this study.

Disclosure statement

The authors declare that they have no conflict of interest.

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