ABSTRACT

Introduction: The aim of this study was to evaluate bone age by means of the Greulich and Pyle method and correlate it with the chronological age in a population sample from the Brazilian northeastern region; sex was also considered.

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**Methods:** This study was conducted after Research Ethics Committee approval. 150 Carpal radiographic images (150) of patients aged 5–18, who were attending a private dental radiology service were evaluated. The images were evaluated by two examiners at two different time intervals. The data were analyzed by means of correlation analyses (Pearson’s $r$), Student's $t$ tests, analysis of variance, and linear regression analyses ($p \leq 0.05$). **Results:** There were strong and positive intra-evaluator and inter-evaluator correlations. The bone and chronological ages were well correlated in both sexes (0.85 female and 0.89 male; $p < 0.001$). For the female sex, the evaluation of bone age was a mean of 8 months higher than the chronological age ($p \leq 0.05$), whereas for the male sex, there was no significant difference ($p = 0.279$). **Conclusions:** Bone age is frequently higher than chronological age, particularly for the female sex. The Greulich and Pyle method using correlation factors is reliable and can be used for estimating age and evaluating the patient's stage of development.

**Key-words:** Skeletal age; hand-wrist radiographs; age determination by skeleton; bone development.

**RESUMEN**

**Introducción:** El objetivo de este estudio fue evaluar la edad ósea mediante el método de Greulich y Pyle y correlacionarlo con la edad cronológica en una muestra de población de la región noreste de Brasil; También se consideró el sexo. **Métodos:** Este estudio se llevó a cabo después de la aprobación del Comité de Ética de Investigación. 150 imágenes radiográficas carpiano (150) de los pacientes de 5-18 años, que asistían a un servicio de radiología dental privado fueron evaluados. Las imágenes fueron evaluadas por dos examinadores en dos intervalos de tiempo diferentes. Los datos fueron analizados mediante análisis de correlación ($r$ de Pearson), Student pruebas $t$, análisis de varianza y análisis de regresión lineal ($p = 0.05$). **Resultados:** Hubo intra-evaluador e inter-evaluador correlaciones fuertes y positivas. Las edades óseas y cronológicas se correlacionan bien en ambos sexos (0.85 femenina y masculina 0,89; $p <0,001$). Para el sexo femenino, la evaluación de la edad ósea fue una media de 8 meses superior a la edad cronológica ($p = 0.05$), mientras que para el sexo masculino, no hubo diferencia significativa ($p = 0.279$). **Conclusiones:** La edad ósea es a menudo mayor que la edad cronológica, en particular para el sexo femenino. El método de Greulich y Pyle usando factores de correlación es fiable y se
puede utilizar para la estimación de la edad y la evaluación de la etapa de desarrollo del paciente.

**Palabras-clave:** Edad ósea; radiografías mano-muñeca; determinación de la edad por el esqueleto; desarrollo de los huesos.
1 INTRODUCTION AND LITERATURE REVIEW

The stage of development of each individual is better estimated considering specific levels of physiological maturity which comprise physiological or developmental indices\textsuperscript{1,2,3}. Skeletal maturity is the most reliable index and is frequently used in clinical routine\textsuperscript{4,5,6}. Skeletal age assessment is used in Pediatric Endocrinology, Neurology, Orthodontics and functional Orthopedics\textsuperscript{7,8} and in Forensic Dentistry\textsuperscript{7}.

It is common to find different bone ages among individuals of the same chronological age, since bone development may be influenced by genetic, racial, climatic, socio-economic, nutritional, environmental and hormonal factors\textsuperscript{1,5,8-12}.

Bone maturity may be followed-up by means of the ossification stages of the hand and wrist bones. This region has various ossification centers and they are processed in parallel to the other areas of the human body\textsuperscript{1,13-16}. Moreover, there is easy access to the region, it presents no risks, and is not uncomfortable for the patient\textsuperscript{8,15}.

Among the various methods proposed for the determination of skeletal age by means of carpal radiographs, the method of Greulich and Pyle is outstanding. This method is based on an inspectional evaluation, comparing the radiograph with the pattern presented in the atlas elaborated in 1950, from a sample of North American children, from birth to the age of 18 years, for the female sex and up to 19 years for the male sex\textsuperscript{17}. It is one of the most used methods for determining the skeletal age of children and adolescents, because it is fast and easy to perform\textsuperscript{9,16,18-21}.

Many studies have evaluated the applicability of the Greulich and Pyle method, and these have been conducted in Central Europe\textsuperscript{22}, Italy\textsuperscript{23}, the USA\textsuperscript{12,24,25}, Turke\textsuperscript{4,10,26}, Denmark\textsuperscript{21}, Taiwan\textsuperscript{5}, Holland\textsuperscript{19}, Pakistan\textsuperscript{7} and in Brazil\textsuperscript{1,13,14,27-30}. Brazilians form one of the most heterogeneous populations in the world, which is the result of five centuries of interethnic crosses of peoples from three continents. There is predominance of Amerindian matrilineages in the Amazon (north) region, a preponderance of African lineages in the northeast, equilibrium in the southeast, and European dominance in the south\textsuperscript{31}. Therefore, it is important to study bone age in the different regions. However, the largest study was conducted in the Southeastern region, particularly in the State of São Paulo\textsuperscript{13,14,27,28,30}. In the Brazilian northeast, there is only the study conducted with a sample in one of the states\textsuperscript{1}.
Therefore, the aim of the present study was to evaluate bone age by means of the Greulich and Pyle method and correlate it with the chronological age, considering sex, in addition to evaluating the reliability of the method in a population sample from the Brazilian northeastern region.

2 MATERIAL AND METHODS
This study was conducted after obtaining approval from the Research Ethics Committee of the University Hospital (Protocol No.208/10). For this study 150 digital radiographs of the hand and wrist of patients from 5 to 18 years of age were evaluated. Of these images, 78 (52%) were of patients of the female sex and 72 (48%) to patients of the male sex. This was a retrospective study, since the carpal images were obtained from an archive of radiographic images of patients attended in the period from April 2009 to April 2010, at a private Dental Radiology Service.

2.1 Obtaining carpal radiographic images
The digital radiographic images of the hand and wrist (Figure 1) were taken using an X-ray appliance Kodak® 8000C (Kodak, Eastman, Rochester, USA), operating at 60kV and 10mA, with an exposure time of 0.2 seconds of exposure with a 2.5mm Al filter. The focus-receptor distance was 1.52 meters. The principles recommended for anthropometry were complied with, and photographs were obtained of the left hand, placed in a centralized position with the palm surface facing down. All the radiographs images were taken using the means of radioprotection and biosafety for preservation of the patient.

The digital radiographs of the hand and wrist were exported and stored at the resolution of 300 dpi, in Tiff format (Tagert Image From File). For evaluation, the images were exported and randomly distributed in the Microsoft Power Point® program (version 2007).
The images were individually evaluated by two examiners in a dark room, with the aid of a 21.5" LED monitor. The examiners were previously trained and had no knowledge about the chronological age of the patients. The digital radiographs of the hand and wrist were compared, considering sex, with the standard image in the atlas of Greulich and Pyle\textsuperscript{17} until there was a coincidence of images (visual method) thus determining the bone age. When the image presented an intermediate pattern between two patterns, a mean was chosen, and the difference in age between some patterns in the atlas was up to 14 months.

Each of the evaluators performed an evaluation of the same image twice, with at least 15 days interval between evaluations. A maximum of 10 images per day were evaluated to avoid visual fatigue.

### 2.2 Data Analysis

The data were recorded in the form of a database in the informatics program SPSS (\textit{Statistical Package for Social Sciences}) for Windows, version 15.0, and analyzed by means of descriptive statistics and bivariate inferential statistics. For the descriptive procedures, central tendency measures were presented (mean and median) and variability (Standard Deviation and Amplitude). In the inferential statistic procedures, when the conditions for the use of parametric statistics were satisfied, correlation analyses (r of Pearson), Student's-\textit{t} tests, analysis of variance (ANOVA)
and linear regression analyses were performed. For all the tests a level of significance of 0.05 was adopted.

3 RESULTS

Table 1 presents the results of intra- and inter-evaluator analyses with regard to the bone ages. There were strong, positive and significant relationships under all the conditions. Considering the explanatory percentage of these correlations (over 94%), a mean bone age was calculated for later analyses.

Table 1 - Results of analyses of intra- and inter-evaluator agreement on bone age by means of the Pearson correlation coefficients.

<table>
<thead>
<tr>
<th>Evaluators</th>
<th>N</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>intra-evaluator 1</td>
<td>150</td>
<td>0.996</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intra-evaluator 2</td>
<td>150</td>
<td>0.977</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inter-evaluators</td>
<td>150</td>
<td>0.970</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*p values ≤0.05 are statistically significant.

In Table 2, the results of the comparative analysis between bone age and chronological age adjusted by sex. There was significant difference for the female sex, in which the mean bone age was almost eight months ahead than the mean chronological age (p≤0.05). For the male sex there was no significant difference between the ages types (p=0.78).

Table 2 - Comparison of chronological age with bone age considering sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Chronological Age</th>
<th>Bone Age</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In months (N (SD))</td>
<td>In months (N (SD))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>134.2 (37.0)</td>
<td>134.8 (44.1)</td>
<td>-0.279</td>
<td>0.78</td>
</tr>
<tr>
<td>Female</td>
<td>122.8 (32.0)</td>
<td>130.4 (37.2)</td>
<td>-3.369</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 3 presents the results of the correlation between bone age and chronological age with respect to sex. There was a correlation between bone age and chronological age of 0.89 for the male sex and 0.84 for the female sex. For males as females, there was significant linear relation between both age and chronological age ($p<0.001$ for both sexes). Table 4 shows the results of the simple linear regression analysis in both sexes and the Graph 1 represents the bone age as a function of chronological age for the female sex.

**Table 3 - Correlation between chronological age and bone age discriminated by sex.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>0.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 4 - Regression equations for bone age discriminated by sex.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Equation</th>
<th>$R^2$ Adjusted (%)</th>
<th>p</th>
<th>CI 95%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>$y= 7.45 + 1.06x$</td>
<td>79</td>
<td>&lt;0.001</td>
<td>0.93 to 1.20</td>
</tr>
<tr>
<td>Female</td>
<td>$y= 9.90 + 0.98x$</td>
<td>70</td>
<td>&lt;0.001</td>
<td>0.84 to 1.12</td>
</tr>
</tbody>
</table>

*CI 95% for regression coefficient.
**Graph 1** - Representation of bone age as a function of chronological age for female sex. Bone age (months) = 9.90 + 0.98 × chronological age (months). $R^2 = 0.70$.

4 DISCUSSION

In the present study a sample of 150 carpal radiographs were used. This number was adopted because it was the total number of images available in the period of two years at a private Dental Radiology service. Moreover, the size of the sample corresponds to a convenience sample, since the large portion of similar studies used a similar number of carpal radiographs, 13, 15, 18, 21, 24, 27, 30. However, other studies used a smaller 7, 8, 22 and larger sample than the present study 1, 4, 5, 10, 11, 14, 19, 29, 32.

As in the study of Van Rijnet al. 19, it was possible to obtain a sample of carpal radiographs of patients with a minimum age of five years. Although there are studies that have used a sample composed of carpal radiographs of patients with an age lower than five years 7, 11, 12, 22, the major portion of the sample was composed of patients with a minimum age of
over five years\textsuperscript{1, 4, 10, 13, 14, 19, 23, 27, 29, 30, 32}. It is difficult to obtain a sample of carpal radiograph images of patients under the age of five, since the carpal radiograph is mostly used to evaluate the maturation status of a child in the diagnosis, treatment planning and eventual outcome in Orthodontics and functional Orthopedics. The follow-up of treatment makes it feasible to obtain carpal radiographs of older patients. The maximum age of the sample in the present study was similar to the majority of previous studies\textsuperscript{4, 7, 10, 11, 12, 19, 22, 23, 29, 32}. However, other studies were composed of a sample of carpal radiographs of patients with a maximum age of up to 16 years\textsuperscript{1, 13, 14, 15, 27, 30}.

To determine the reliability of the method, the capacity of the same evaluator to produce his results in a time interval and the correlation of the evaluations performed by two evaluators of the same radiographs was observed. The results showed strong and significant correlation of both intra-evaluators and inter-evaluators, confirming the reproducibility of the method. Similar results have been found by other researchers\textsuperscript{5, 8, 11, 19, 21, 23, 30}. These results emphasize that the Greulich and Pyle method is easy to perform, and is widely used all over the world. In the present study the high correlation found resulted from the training before the analyses of the radiographs, when it was sought to establish a pattern or sequence of evaluation, in addition to the ease and simplicity of performing the method.

In the present study, the $R^2$ value of 0.79 for the female sex and 0.70 for the male, indicated that 79% and 70% of the bone age can be predicted by the chronological age respectively. This result represents good correlation between bone age and chronological age for both sexes. The correlation indices obtained were similar to those observed by other authors\textsuperscript{1, 4, 8, 13, 19, 30}. Furthermore, it is pointed out that in a Korean population, of the three methods of evaluation, the Greulich-Pyle method was the one that obtained the best correlation index. However, in an Italian population sample with an age-range between 11 and 19 years, there was a large margin of error in the determination of chronological age with the Greulich-Pyle method, particularly in the determination of the ages of 14 and 18 years, so that this method is not indicated for helping to determine chronological age in forensic researches\textsuperscript{23}.

Therefore, one must be aware of the applicability of the Greulich-Pyle method in distinct populations, because some factors are known to influence the development of individuals, such as genetics, race, nutrition, hormonal, environment, socioeconomics, culture and sex\textsuperscript{1, 5, 9, 10, 11, 25}. 


In the present study both sexes presented higher bone ages in relation to chronological ages, demonstrating advanced maturity. Higher bone ages than chronological ages for both sexes were also observed in Turkish children\(^4\), Central European children\(^22\) and in children in the southeast of Brazil\(^14\). On the other hand, some authors have found overestimation of bone age only in the female sex\(^13, 26, 30\), and two of these researches\(^13, 26\) used a sample from the southeast of Brazil.

Girls usually exhibit earlier bone development than boys\(^28\). In the present study that was more precocity for the female sex, which demonstrates the distinct behavior between the sexes, being similar to the data found in a study conducted in the southeast of Brazil\(^28\). Conversely, in a study also using a sample from the southeast of Brazil, there were no significant differences in bone age between the sexes\(^1\). Moreover, there is disagreement with the result of the present study with regard to the data found in a sample in the southeastern region in Brazil, \(^14, 27\) and in Slovenians\(^19\). In these studies there were delays in bone age in relation to chronological age in both sexes. Furthermore, it is emphasized that delayed bone ages before puberty were noted in children in Taiwan\(^5\), Turkey\(^10\) and Pakistan\(^7\).

Although the correlations between bone age and chronological age had also been good, the use of correlation factors is recommended for better adaptation of the method to the studied population, since it presents different characteristics to the population for which the atlas was developed\(^1, 13, 30\). Therefore, regression equations were created for each sex, because as a datum of development, bone age must be referred to the patterns of the collective society to which the individual belongs. The regression analysis allows one to estimate by means of equations, the extent to which bone age varies in relation to chronological age and vice versa. As the intervals of confidence suggested different variabilities, the regression analyses were discriminated by sex. Thus, an equation was obtained for the male sex and another for the female sex.

The difference observed between the ages in the present study demonstrated that children within the same age range develop in an unequal manner. In the clinic, it allows us to reflect on the importance of determining the correct stage of an individual's development, in which the ideal time of treatment must consider each case in particular. The possibility of predicting growth is an auxiliary tool in the diagnosis, prognosis and elaboration of the treatment plan. In cases of providing expert opinion, underestimating or overestimating age may favor or
prejudice the individual. Therefore, it is important to use the factors of correction to estimate the patient's age or to evaluate his/her stage of development, and differences between the sexes must be considered.

5 CONCLUSIONS

In the studied sample the Greulich and Pyle method was shown to be reliable, presenting correlation between the bone and chronological age for both sexes. From the differences found with regard to the different regions of Brazil and other countries, it is most important that this method should be applied using the correction factors by means of previous studies in the populations. Therefore, the applicability of the Greulich and Pyle method is a valid tool for its application in Forensic Dentistry, determination of chronological age, and in Orthodontics and Orthopedics for the determination of bone maturity.

REFERENCES


