

## Comparison among dental, skeletal and chronological development in HIV-positive children: a radiographic study

### *Comparação entre o desenvolvimento dentário, ósseo e cronológico em crianças HIV<sup>+</sup>: estudo radiográfico*

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**ABSTRACT:** The goal of this study was to evaluate skeletal, dental and chronological development in an HIV-positive group of children, as compared with a control group, during a four-year period. Panoramic radiographs and hand and wrist radiographs of 60 children were taken. The children, of both sexes, aged 5 years and 2 months to 15 years and 5 months, were selected as follows: 30 HIV-positive volunteers who had acquired the disease vertically, and 30 volunteers who did not present the HIV infection or any other systemic disease. All radiographs were technically standardized and analyzed according to criteria established by Nolla (dental age), Greulich and Pyle (bone age), and Eklöf and Ringertz (bone age). The results were submitted to Student's *t*-test at a 5% level of significance. Based on the comparison between the chronological age and the dental or the skeletal age, significant differences were observed between HIV-positive and HIV-negative children, both in 1999 and in 2003 ( $p \leq 0.05$ ). Considering the results obtained with the methodology used, it was concluded that HIV-positive children of both sexes presented delayed bone development despite the administration of antiretroviral drugs, and that HIV-positive female children presented younger dental ages compared with their chronological ages in 1999 and in 2003; and HIV-positive males, in 1999.

**DESCRIPTORS:** Age determination by skeleton; Age determination by teeth; HIV; Acquired immunodeficiency syndrome.

**RESUMO:** Este trabalho teve como objetivo avaliar, em um intervalo de quatro anos, o desenvolvimento ósseo, dentário e cronológico de um grupo de crianças portadoras do HIV, comparando-o com um grupo controle. Foram realizadas radiografias panorâmica e de mão e punho, de uma amostra de 60 crianças, com idades variando entre 5 anos e 2 meses e 15 anos e 5 meses, dos sexos feminino e masculino, sendo: 30 crianças, voluntárias, portadoras de infecção pelo HIV, adquirida verticalmente, e 30 crianças voluntárias, que não apresentavam infecção pelo HIV ou qualquer outra doença sistêmica. Todas as radiografias foram padronizadas tecnicamente e analisadas segundo os critérios dos métodos de Nolla (idade dentária), Greulich e Pyle (idade óssea) e Eklöf e Ringertz (idade óssea). Os resultados foram submetidos ao teste estatístico *t*-Student em um nível de significância de 5%. Verificou-se, através da comparação da idade cronológica com as idades dentária e óssea, que houve diferença entre as crianças HIV<sup>+</sup> e as não portadoras do vírus, tanto em 1999 como em 2003 ( $p \leq 0,05$ ). Concluiu-se que as crianças HIV<sup>+</sup> deste estudo sofreram um retardo no desenvolvimento ósseo. A idade dentária foi inferior à idade cronológica no grupo feminino HIV<sup>+</sup>, em 1999 e 2003, e masculino HIV<sup>+</sup> em 1999.

**DESCRIPTORIOS:** Determinação da idade pelo esqueleto; Determinação da idade pelos dentes; HIV; Síndrome de imunodeficiência adquirida.

## INTRODUCTION

Before 1983, it was believed that AIDS (acquired immunodeficiency syndrome) was restricted to a specific group of people. In that year, however, the first cases were reported of the syndrome affecting children<sup>14</sup>. Among children, most cases of infection by HIV are transmitted vertically, i.e.,

from mother to child. Newborn children present an immature immune system, which may cause the rapid and severe progression of the disease<sup>1</sup>.

Children who are vertically infected present considerable weight loss and delayed development<sup>2,5,9,12,15</sup>. They may also present the following

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changes: delayed dental eruption, fewer permanent teeth, prolonged retention of primary teeth<sup>16</sup> and a slightly larger number of dental anomalies<sup>6</sup>. The establishment of antiretroviral protocols has reduced the occurrence of oral changes<sup>7,10</sup>. However, some authors have reported delayed development even with the use of antiretroviral drugs<sup>17</sup>.

In this study, the assessment of dental and skeletal growth and development was performed by means of significant parameters of skeletal maturation and dental development. The stages of dental calcification were assessed according to the method proposed by Nolla<sup>13</sup> (1960). Skeletal development was assessed according to the methods proposed by Greulich, Pyle<sup>8</sup> (1959) and Eklöf, Ringertz<sup>4</sup> (1967).

Recently, the digitalization of radiographic images has been performed to obtain greater accuracy with these methods. Linear measurements have also been made using a specific computer program, to demonstrate the level of bone development<sup>3,11</sup>.

The aim of this study was to carry out a longitudinal analysis of skeletal and dental development in HIV<sup>+</sup> children and in control children during a period of 4 years.

## MATERIAL AND METHODS

A total of 60 children, aged 5 years and 2 months to 11 years and 8 months, in the first phase of the research (1999), and 8 years and 5 months to 15 years and 5 months, in the second phase of the research (2003), participated in this study. Among the children, 30 were HIV-positive volunteers, 17 male and 13 female, who had been vertically infected and were under outpatient treatment at the Pediatric service of the Clinical Hospital, Federal University of Rio Grande do Sul, Porto Alegre; and 30 were HIV-negative volunteers, 17 male and 13 female, who did not present any other systemic condition, and were seen at the School of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre. The HIV-negative children presented chronological ages similar to those of the HIV-positive children in both phases of the study, and subjects were paired according to the following criteria: age, socioeconomic level, gender and race. All HIV-positive children were under antiretroviral therapy.

The children were examined and radiographed from January 1999 to October 1999, in phase 1, and from December 2002 to September 2003, in phase 2. They were submitted to routine clinical

examination at the School of Dentistry, PUCRS, and panoramic radiographs, as well as hand and wrist radiographs, were taken. All radiographs were technically standardized using the same equipment (Orthophos Siemens, Munich, Germany) and processed using automatic processor AT2000 (Air Techniques, Hicksville, New York, USA). During the radiographic take, patients used a long lead apron for protection. Analysis of the radiographs was performed by a single radiologist at 3 different times with a 10-day interval between readings.

The study was approved by the Research Ethics Committees of the Clinical Hospital of Porto Alegre (HCPA) and of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

### Dental age assessment – Nolla method

Dental age assessment was performed by analyzing the dental calcification stages observed in the panoramic radiographs according to the method established by Nolla<sup>13</sup> (1960). The teeth analyzed were the mandibular left permanent teeth. Each tooth was classified according to its calcification stage and the values were added up, resulting in one single value per child. When teeth were found to be between 2 stages, an average value was assigned.

### Bone age assessment – Greulich and Pyle method

Each hand and wrist radiograph was compared with the atlas pattern of similar age and sex. Older and younger patterns were also observed<sup>8</sup>. Bones and their epiphyses were then analyzed following a standardized sequence: distal surfaces of the radius and ulna bones, carpal bones (capitate, hamate, triquetral, lunate, scaphoid, trapezium, trapezoid and pisiform bones), metacarpal bones and distal phalanges. In cases where there was a sesamoid bone, its presence was also recorded, since it indicates the beginning of puberty.

### Bone age assessment – Eklöf and Ringertz method

This method measures the distances that correspond to the width and length of the hand and wrist bones, according to 10 parameters established by Eklöf, Ringertz<sup>4</sup> (1967). To perform this analysis, the radiographs were scanned using a flatbed scanner with transparency module (SnapScan TPO, AGFA, Shanghai, People's Republic of China), and assessed using the Image

Tool program for digitalized images (UTHSCSA, Texas, USA). The data obtained were tabulated and statistically analyzed by means of Student's *t*-test for paired samples at a 5% significance level ( $p \leq 0.05$ ).

## RESULTS

### Female sex

Based on the Greulich and Pyle, and the Nolla methods, the values observed for the HIV-positive group were significantly lower than those observed for the control group in 1999 ( $p \leq 0.01$ ). In 2003,

significant differences were also observed between the two groups according to the methods established by Greulich and Pyle ( $p \leq 0.02$ ) and by Eklöf and Ringertz ( $p \leq 0.01$ ); moreover, the values obtained for the HIV-positive group were lower than those obtained for the control group (Table 1).

According to the Greulich and Pyle, and the Nolla methods, HIV-positive females, in 1999, presented significantly older chronological age than the standard ages described by these authors ( $p \leq 0.01$ ) (Table 2).

In 2003, HIV-positive female children presented older chronological ages than those obtained by

**TABLE 1** - Comparison among the ages (years) obtained for HIV<sup>+</sup> and control groups - Female sex - 1999 and 2003.

Comparison		n	Average	Standard-deviation	p
Greulich and Pyle	HIV <sup>+</sup> group-1999	13	5.25	1.46	0.01*
	Control group-1999	13	7.46	2.10	
	HIV <sup>+</sup> group-2003	13	9.67	1.58	0.02*
	Control group-2003	13	10.91	1.62	
Eklöf and Ringertz	HIV <sup>+</sup> group-1999	13	7.32	1.19	0.96
	Control group-1999	13	7.34	1.42	
	HIV <sup>+</sup> group-2003	13	10.42	1.08	0.01*
	Control group-2003	13	11.13	1.59	
Nolla	HIV <sup>+</sup> group-1999	13	6.24	1.24	0.01*
	Control group-1999	13	7.52	1.39	
	HIV <sup>+</sup> group-2003	13	9.78	1.38	0.18
	Control group-2003	13	10.39	1.25	

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

**TABLE 2** - Comparison of the chronological ages and the ages (years) according to the methods of Greulich, Pyle<sup>8</sup> (1959), Eklöf, Ringertz<sup>4</sup> (1967), and Nolla<sup>13</sup> (1960) - HIV<sup>+</sup> and control groups - female sex - 1999.

Comparison		n	Average	Standard-deviation	p
HIV <sup>+</sup> -1999	Chronological age	13	7.36	1.45	
	Greulich and Pyle	13	5.25	1.46	0.01*
	Eklöf and Ringertz	13	7.32	1.19	0.94
	Nolla	13	6.24	1.24	0.01*
Control-1999	Chronological age	13	7.32	1.43	
	Greulich and Pyle	13	7.46	2.10	0.66
	Eklöf and Ringertz	13	7.34	1.42	0.73
	Nolla	13	7.52	1.39	0.09

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

Greulich, Pyle<sup>8</sup> (1959) and Nolla<sup>13</sup> (1960) ( $p \leq 0.01$ ) and by Eklöf, Ringertz<sup>4</sup> (1967) ( $p \leq 0.02$ ). In the control group, the chronological age was significantly older than that obtained according to the Nolla method ( $p \leq 0.01$ ) (Table 3).

### Male sex

Among males of the HIV-positive and control groups, in 1999, a significant age difference was observed based on the Greulich and Pyle, and the Nolla methods. Values obtained for the HIV-positive group were lower than those obtained for the

control group. No significant difference between the two groups was observed according to the Eklöf, Ringertz<sup>4</sup> (1967) method. In 2003, no statistically significant differences were observed between the two groups ( $p > 0.01$ ) (Table 4).

In 1999, HIV-positive patients presented significantly older chronological ages than those observed according to the Greulich, Pyle<sup>8</sup> (1959) and the Eklöf, Ringertz<sup>4</sup> (1967) methods ( $p \leq 0.01$ ). According to the Nolla method, no significant difference between chronological and dental ages was observed. The control group presented a signifi-

**TABLE 3** - Comparison of the chronological ages and the ages (years) according to the methods of Greulich, Pyle<sup>8</sup> (1959), Eklöf, Ringertz<sup>4</sup> (1967), and Nolla<sup>13</sup> (1960) – HIV<sup>+</sup> and control groups – female sex – 2003.

Comparison		n	Average	Standard-deviation	p
HIV <sup>+</sup> -2003	Chronological age	13	10.96	1.45	
	Greulich and Pyle	13	9.67	1.58	0.01*
	Eklöf and Ringertz	13	10.42	1.08	0.02*
	Nolla	13	9.78	1.38	0.01*
Control-2003	Chronological age	13	11.14	1.56	
	Greulich and Pyle	13	10.91	1.63	0.21
	Eklöf and Ringertz	13	11.13	1.59	0.96
	Nolla	13	10.39	1.25	0.01*

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

**TABLE 4** - Comparison among the ages (years) obtained for the HIV<sup>+</sup> and control groups – male sex – 1999 and 2003.

Comparison		n	Average	Standard-deviation	p
Greulich and Pyle	HIV <sup>+</sup> group-1999	17	5.67	2.32	0.01*
	Control group-1999	17	7.06	2.51	
	HIV <sup>+</sup> group-2003	17	10.08	3.10	0.09
	Control group-2003	17	11.04	2.12	
Eklöf and Ringertz	HIV <sup>+</sup> group-1999	17	6.92	2.36	0.26
	Control group-1999	17	7.09	2.02	
	HIV <sup>+</sup> group-2003	17	10.70	2.42	0.96
	Control group-2003	17	11.71	2.22	
Nolla	HIV <sup>+</sup> group-1999	17	6.90	2.26	0.01*
	Control group-1999	17	7.64	2.30	
	HIV <sup>+</sup> group-2003	17	11.07	1.80	0.07
	Control group-2003	17	11.84	2.62	

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

**TABLE 5** - Comparison of the chronological ages and the ages (years) according to the methods of Greulich, Pyle<sup>8</sup> (1959), Eklöf, Ringertz<sup>4</sup> (1967), and Nolla<sup>13</sup> (1960) – HIV<sup>+</sup> and control groups – male sex – 1999.

Comparison		n	Average	Standard-deviation	p
HIV <sup>+</sup> -1999	Chronological age	17	7.26	2.36	
	Greulich and Pyle	17	5.67	2.32	0.01*
	Eklöf and Ringertz	17	6.92	2.36	0.01*
	Nolla	17	6.90	2.26	0.07
Control-1999	Chronological age	17	7.30	2.27	
	Greulich and Pyle	17	7.06	2.51	0.20
	Eklöf and Ringertz	17	7.09	2.02	0.08
	Nolla	17	7.64	2.30	0.02*

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

**TABLE 6** - Comparison of the chronological ages and the ages (years) according to the methods of Greulich, Pyle<sup>8</sup> (1959), Eklöf, Ringertz<sup>4</sup> (1967), and Nolla<sup>13</sup> (1960) – HIV<sup>+</sup> and control groups – male sex – 2003.

Comparison		n	Average	Standard-deviation	p
HIV <sup>+</sup> -2003	Chronological age	17	11.62	2.56	
	Greulich and Pyle	17	11.04	2.12	0.01*
	Eklöf and Ringertz	17	10.71	2.22	0.01*
	Nolla	17	11.84	2.62	0.44
Control-2003	Chronological age	17	11.28	2.53	
	Greulich and Pyle	17	10.08	3.11	0.03*
	Eklöf and Ringertz	17	10.70	2.42	0.01*
	Nolla	17	11.07	1.80	0.53

\*Significantly different. Source: School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - RS.

cantly older dental age compared with chronological age in 1999 ( $p \leq 0.02$ ) (Table 5).

In 2003, HIV-positive patients presented significantly older chronological ages than those observed according to the Greulich and Pyle, and the Eklöf and Ringertz methods ( $p \leq 0.01$ ). The control group also presented significantly older chronological ages than those observed according to the methods established by Greulich and Pyle ( $p \leq 0.03$ ) and by Eklöf and Ringertz ( $p \leq 0.01$ ) (Table 6).

## DISCUSSION

The first reports of HIV infection in children date back to 1983, when Oleske *et al.*<sup>14</sup> reported the different characteristics observed in children when compared with adults, including the transmission pattern. HIV infection may cause

deficient bone maturation<sup>9,17</sup> and delayed dental development<sup>6,16</sup>. In the present study, when HIV-positive and HIV-negative children of both sexes were compared using the methods proposed by Nolla<sup>13</sup> (1960), by Greulich, Pyle<sup>8</sup> (1959) and by Eklöf, Ringertz<sup>4</sup> (1967), delayed skeletal and dental development was also observed. The present study revealed that, both in 1999 and in 2003, HIV-positive females presented younger skeletal and dental ages when compared with their chronological ages. However, the difference between control and HIV-positive patients was greater in 1999. This indicates that the medication given to HIV-positive patients was a favorable factor<sup>1,2</sup>.

In 1999, HIV-positive males presented significantly younger skeletal and dental ages than those of the control group. These results are consistent with the findings of Fine *et al.*<sup>7</sup> (2003).

According to the Nolla method, the HIV-positive female group presented a deficiency in dental development in 1999, as did both the HIV-positive and control groups in 2003. The same happened to the HIV-positive male group in 1999, whereas the control group presented older dental age compared with their chronological age.

Sierra<sup>18</sup> (1987) and Vallejo-Bolaños *et al.*<sup>19</sup> (1999) also observed significant differences between chronological and dental ages, although using different methods. Fine *et al.*<sup>7</sup> (2003) showed that HIV-positive children present prolonged retention of primary teeth and delayed tooth eruption when compared with non-infected children. These findings, similar to the results of our study, may indicate impairment to normal development. Ramos-Gomez *et al.*<sup>16</sup> (2000) reported a smaller number of erupted teeth in HIV-positive children. However, Fernandes *et al.*<sup>6</sup> (2002) found no significant differences between HIV-positive and HIV-negative children. Laskaris<sup>10</sup> (2000) suggested that antiretroviral drugs may have caused a decrease in the number of oral alterations. This could explain why some HIV-positive children presented dental development similar to that of non-infected children.

According to the Greulich and Pyle method, HIV-positive female patients presented older chronological ages compared with their bone ages in 1999 and in 2003. According to the Eklöf and Ringertz method, HIV-positive female patients presented older chronological ages compared with their bone ages only in 2003. This indicates a deficiency in bone growth. The same was observed for both the male HIV-positive and the control groups.

Moye *et al.*<sup>12</sup> (1996) and Abuzaitoun, Hanson<sup>1</sup> (2000) reported that an immature immune

system such as that of children may cause deficient organic growth and development, especially in HIV-positive children. This explains why, in this study, most HIV-positive children presented younger bone ages when compared with their chronological ages. However, in this study, little difference was observed between results for HIV-positive and HIV-negative children. This may be explained by the fact that the former were undergoing antiretroviral therapy, as demonstrated by Arpadi<sup>2</sup> (2000); Abuzaitoun, Hanson<sup>1</sup> (2000). Our results are corroborated by Oliveira<sup>15</sup> (2002), who observed the influence of the HIV virus on skeletal development in female children. Even though the European Collaborative Study<sup>5</sup> (1995) and Moye *et al.*<sup>12</sup> (1996) reported a strong correlation between vertically-acquired infection and growth deficiency, the results of the present study do not completely corroborate this fact.

Adequate antiretroviral therapy may be an important factor to reestablish normal development in HIV-positive children. Further studies must be carried out to evaluate children whose diagnosis and therapy occurred early at birth and children whose diagnosis was confirmed only after the appearance of the first signs and symptoms of HIV-infection.

## CONCLUSIONS

Considering the results obtained with the methodology used, it was concluded that HIV-positive children of both sexes present delayed bone development despite the administration of antiretroviral drugs, and that HIV-positive female children present younger dental ages compared with their chronological ages in 1999 and in 2003; and HIV-positive males, in 1999.

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