Serum Alkaline Phosphatase Levels in Healthy Children and Evaluation of Alkaline Phosphatase z-scores in Different Types of Rickets

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Introduction

Alkaline phosphatases (ALP) are a group of cell membrane metalloenzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate (1). ALP is expressed mainly in bone, liver, intestines, proximal convoluted tubules of the kidney, and in the placenta. ALP released from these tissues constitutes the total amount measured in the blood. Total ALP activity changes with age and bone fraction, varying from 77% to 89% in children and from 58% to 67% in adults (3,4,5). In contrast to ALP isoenzymes, total serum ALP is widely used in routine biochemical tests and can be performed in almost all laboratories. Traditionally, total serum ALP activity has been used as a biochemical marker for bone formation to assess osteoblastic activity in primary hyperparathyroidism, rickets, osteomalacia and Paget’s disease. Since ALP is a marker for osteoblastic activity, growing children have higher levels than fully grown individuals. Highest levels of ALP are detected during the rapid growth phases of childhood such as infancy and puberty (2,6,7,8). While it is known that reference values of serum ALP are highly dependent on age and sex in childhood, most commercial assay kits used in hospital laboratories provide no specific references are given for pediatric ages. Additionally, the colorimetric method using p-nitrophenyl phosphate as substrate is the method used by most clinical laboratories, but the buffer varies between assays. 2-amino-2-methyl-1-propanol (AMP) is the buffer recommended by both the American Association for Clinical
Chemistry (AACC) (9) and the International Federation of Clinical Chemistry (IFCC) (10); this buffer is also used in the method described by Breuadie et al (11). On the other hand, diethanolamine (DEA) is recommended by the Scandinavian Society for Clinical Chemistry (SSCC) (12). Higher values were obtained with the SSCC method (12), owing to the increased sensitivity resulting from the use of DEA buffer. The lower values with the AACC/IFCC method (9,10) can be attributed to a lower concentration determined with the AMP buffer. Consequently, there are great variations in serum ALP levels assayed in different laboratories and in the interpretation of the results. Therefore, it is necessary to state the buffer used in the assay when reporting the ALP levels. This is particularly important for the pediatric age group, in which the upper limit of reference ranges may show an almost twofold variation.

In this study, we aimed to establish pediatric age- and sex-specific reference ranges for serum total ALP by the colorimetric method using p-nitrophenyl phosphate as substrate and diethanolamine as buffer and run by Roche/Hitachi 917/MOD system. Additionally, we compared the ALP z-scores of patients with different types of rickets by using these reference ranges.

Methods

Sample Selection

We studied 1922 children aged 0 to 18 years who presented to the outpatient clinic for routine check-ups or for minor conditions requiring analysis of blood samples for diagnosis. Children who showed clinical signs of any acute or chronic illnesses affecting serum ALP levels were not included in the study. Children and adolescents under treatment with corticosteroids or anticonvulsants, those with neuromuscular disorders or movement impairment, and those with genetic syndromes or major congenital malformations were also excluded. Additionally, serum parathormone (PTH) levels were measured in children with ALP levels in the higher quartile according to their age groups and the children with high PTH levels were excluded from the study.

The Ethics Committee of the Medical Faculty at the Marmara University approved the study. Informed consent was obtained from the parents of each child and from the child if older than 16 years of age. The study was performed between March 2003 and April 2006.

1741 children (904 girls and 837 boys) were included in the final analysis after excluding subjects who were found to have clinical or laboratory evidence of conditions, which might affect the serum ALP levels. The age- and sex-specific reference ranges for serum ALP levels were constructed from the data of these 1741 children.

Additionally, serum ALP levels of 77 different samples from 53 patients with active rickets were analyzed to determine the ALP z-scores in different types of rickets and validate the constructed reference data. This group included 39 samples from 38 children with nutritional rickets (NR) (27 males and 11 females), 19 samples from 7 (3 males, 4 females) with vitamin D-dependent rickets (VDDR) and 19 samples from 8 (3 males, 5 females) with hypophosphatemic rickets (HR).

Statistical analyses were performed using Jandel SigmaStat version 2.0. The unpaired t-test was used to compare ALP levels between girls and boys in different age groups. Kruskal-Wallis one-way analysis of variance by ranks was used to compare ALP z-scores, PTH, calcium and phosphate levels between the different types of rickets.

Results

Serum ALP levels were highest in the first 6 months of life, decreased to relatively steady levels thereafter and started to increase again after age 9 years (Figure 1). The pubertal peak levels were not as high as the levels detected in the first 6 months of life. ALP levels (+2SD) exceeding 900 U/L were observed in two age groups in girls, namely 0-6 months and 12-13 years. In boys, these high levels were observed in six age groups, namely 0-6 months, 6-12 months, 1 year, 10-11 years, 12-13 years and 14-15 years (Table 1). Serum ALP levels were similar in boys and girls until age 10 years, but higher ALP levels were noted at ages 10-11 years in girls (median values were 572 and 525, respectively; p= 0.02) and in three age groups, namely 12-13, 14-15 and 16-17 years in boys (median values were 630 U/L, 518 U/L, 587 U/L, 251 U/L, 369 U/L and 167 U/L, respectively; p<0.001). ALP levels start to decline after 12 years of age in girls and after 14 years of age in boys, with upper ranges approaching the upper ranges of adults (240 U/L for females, 270 U/L for males) in girls (293 U/L), but not in boys (430 U/L) until they reach the ages of 16 to 18 years.

Serum ALP Levels in Children with Rickets

The serum ALP levels from patients with NR prior to treatment were analyzed according to normative data obtained in the study. Additionally, repeated measurements were
performed in the groups of VDDR and HR before treatment as well as after the start of treatment if they continued to have signs of active rickets related to compliance or other problems at follow-up. A total of 39 samples from 38 cases with NR, 19 samples from 7 cases with VDDR, and 20 samples from 8 cases with HR were obtained for final analysis. The biochemical data of patients with rickets are presented in Table 2. Serum ALP levels were highest in the VDDR group and lowest in the HR group. ALP z-score was significantly lower in the HR group (median: 3.6) than in the VDDR (median: 10.4) and NR (median: 6.5) groups (p<0.001). A similar pattern was observed for plasma PTH levels from highest to lowest in VDDR, NR and HR (median values of 525, 237 and 98 pg/mL, respectively). There were statistically significant differences among the three groups (p<0.001). Serum calcium levels were highest and serum phosphate levels were lowest in the HR group (HR vs. VDDR and NR; medians: 9.4 vs. 8.9 and 8.8 mg/dL for Ca, 2.5 vs. 2.9 and 2.9 mg/dL for phosphate, respectively; p<0.05). Serum ALP levels of the individual patients are shown in Figure 2.

**Discussion**

In the present study, we described age- and sex-specific serum ALP levels for ages 0 to 18 years. Serum ALP levels were determined by a widely used colorimetric assay, with p-nitrophenyl phosphate as substrate and diethanolamine as buffer. Serum ALP levels were also performed in patients with different types of rickets and were compared with the normative data.

![Table 1. Mean serum ALP levels (U/L), -2SD, +2SD and square root transformed mean and SD values by age groups](image)
In healthy children, normal ALP levels showed a tetraphasic pattern with highest levels in infancy and puberty and troughs at mid-childhood and at the end of puberty. ALP levels were similar during childhood in both sexes, but showed a dimorphic pattern after the age of 10. Studies evaluating bone-specific ALP levels demonstrate that 77% to 89% of the total ALP levels in children are of bone origin. The studies also indicate that the increase in ALP in pubertal ages reflects the rapid growth in puberty (3,4,5,7,13). Although, we did not check the pubertal status of the children in this study, we can clearly see the pubertal effect through the differences between boys and girls during the pubertal ages. As a result of earlier onset, earlier growth spurt and earlier completion of puberty, most girls in the 16-18 years age group reached ALP levels that were compatible with the upper ranges of adult females. Boys appear to reach the adult levels at later ages. These results confirm the previous data emphasizing that serum ALP levels decline to the adult ranges after the age of 20 in boys and ages 17-18 years in girls (2,7).

The mean ALP levels obtained in this study were similar to the references, in which DEA is used as a buffer (6,7,8), but higher than the values in the references using AMP as buffer (2,14). Different normative data according to the methodology necessitate development and use of z-scores when reporting ALP levels in clinical studies and case reports. This important issue is usually neglected. Thus, we also analyzed serum ALP levels in 3 different types of rickets and, to our knowledge, reported for the first time ALP z-scores for three different types of rickets patients. The highest ALP levels were detected in patients with VDDR and the lowest ones in HR patients; this pattern was valid also for PTH levels. Serum calcium levels were highest and, as expected, serum phosphate levels were lowest in HR patients. Furthermore, it is not possible to compare our data with ALP levels reported in the literature since there is no mention of buffers used for ALP measurement in rickets patients in most reports. We believe that using ALP z-score in the follow-up of patients, instead of using absolute ALP levels, would be more logical and free of age- and sex-related physiological changes in ALP levels and will give a better idea about the pathological process.

**Table 2.** Serum calcium, phosphate, plasma parathyroid hormone levels with serum ALP levels and ALP z-scores in patients with different types of rickets. Mean±SD values are given.

|                  | NR (n=38) | VDDR (n=7) | HR (n=8) | Reference Ranges |
|------------------|-----------|------------|----------|------------------|
| Age (years)      | 1.5±1.3   | 4.7±4.4    | 9.2±5.5  |                  |
| Ca (mg/dL)       | 7.8±1.5\* | 8.8±0.8\*  | 9.4±0.6\* | 8.4-10.5         |
| P (mg/dL)        | 3.6±1.4\* | 3.3±0.9\*  | 2.6±0.5\* | 3.8-6.5          |
| (1-3 years)      |           |            |          | 3.7-5.6          |
|                  |           |            |          | (4-11 years)     |
|                  |           |            |          | 2.9-5.4          |
|                  |           |            |          | (12-15 years)    |
| PTH (pg/mL)      | 323±218*  | 742±634*   | 120±71*  | 9-52             |
| ALP (U/L)        | 2422±1098 | 3377±2052  | 1197±387 |                  |
| ALP z-score (range) (U/L) | 7.1±3.8* | 11.2±6.1*  | 4.2±1.6*  | (2.3-18.0)       |
| ALP z-score (range) (U/L) | (2.4-23.5) | (2.1-7.4) |          |                  |
| Number of samples | 39        | 19         | 20       |

*p<0.001, between three different group
\*p<0.05, HR vs. VDDR and HR vs. NR
Ca: calcium, P: phosphate, PTH: parathormone, ALP: alkaline phosphatase, NR: nutritional rickets, VDDR: vitamin-D dependent rickets, HR: hypophosphatemic rickets

**Figure 1.** Serum ALP levels according to age showing a tetraphasic course from birth to adulthood

**Figure 2.** ALP levels in different types of rickets, full circles representing NR, stars VDDR and empty circles HR

ALP: alkaline phosphatase, NR: nutritional rickets, VDDR: vitamin-D dependent ricket, HR: hypophosphatemic rickets
Additionally, not only normative upper limits of ALP, but also the lower ones are of interest in clinical settings, especially in hypophosphatasia patients. Hypophosphatasia is characterized by low serum ALP activity (hypophosphatasemia) due to loss-of-function mutation within TNSALP, the gene that encodes "tissue-nonspecific" ALP [15,16,17].

We hope that this normative data for ALP levels will be useful in establishing the diagnosis and monitoring the treatment in patients with abnormal ALP levels. Determination of z-scores for ALP will allow more precise assessment of changes in ALP levels in rickets and other bone disorders.

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