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Cross-sectional analysis: clinical presentation of children with persistently low ALP levels

https://doi.org/10.1515/jpem-2021-0330
Received May 11, 2021; accepted August 6, 2021;
published online August 23, 2021

Abstract

Objectives: Low activity of serum alkaline phosphatase (ALP) is a hallmark of hypophosphatasia (HPP), but low readings of ALP are not always recognized in clinical routine. Understanding the clinical presentations associated with low ALP may contribute to a timelier diagnosis of HPP.

Methods: Data from paediatric patients with low ALP, excluding patients in intensive care and with oncological/haematological disorders, were analysed. Most recent ALP values, previous diagnoses, medication and relevant symptoms were extracted from patient records at nine specialised centres and analysed descriptively. A relationship between body height and ALP values was scrutinised by linear regression.

Results: Of 370 children, 15 (4.1%) had a diagnosis of HPP. In the subgroup without a diagnosis of HPP, 241 (67.9%) out of 355 patients had one or more medical conditions known to be associated with low serum ALP. Of those, hypothyroidism, malnutrition and steroid administration were most frequent. Characteristic symptoms, particularly, short stature, muscle weakness and delay of motor development were more frequent and ALP values were lower in patients with documented HPP diagnosis compared to patients without diagnosis of HPP (Ø z-scores: −2.52) (interquartile range [IQR] = 0.20) vs. −1.96 (IQR = 0.87). A weak positive linear relationship between z-scores of ALP and body height was identified ($p<0.001$).

Conclusions: This analysis of paediatric patient records elucidates a wide range of disorders associated with low ALP activity. In case of additional specific symptoms, HPP should always be considered as a differential diagnosis.

Keywords: alkaline phosphatase; bone mineralisation disorder; clinical findings; hypophosphatasia.

Introduction

Alkaline phosphatase (ALP) is a membrane-bound ubiquitous phosphomonoesterase that catalyses the hydrolysis of phosphate monoesters [1–3]. In humans, four isoenzymes of ALP are encoded by separate genes with three tissue-specific – intestinal, placental and germ-cell – isoforms and the ubiquitously expressed tissue-non-specific alkaline phosphatase (TNSALP) [1, 4]. TNSALP is most abundant in the bone, liver, kidney and deciduous teeth and accounts for about 95% of the total ALP activity in the serum [1, 5]. TNSALP hydrolyses inorganic pyrophosphate (PPi), which is a natural inhibitor of bone mineralization. The enzyme is therefore essential for bone and tooth mineralisation [3, 6, 7].

ALP levels vary considerably with age and are highest during increased growth in childhood and adolescence [8–10]. Unlike elevated serum levels of ALP being an established marker of multiple pathologic conditions, low serum ALP activity is less frequently encountered in clinical practice and is not always recognised. Thus, low ALP readings may be overlooked and potentially lead to a diagnostic delay in patients with hypophosphatasia (HPP), adding to the burden of the disease [11–13].

Persistently low serum ALP activity is a biochemical hallmark of HPP [14]. The disease is caused by lack or deficiency of TNSALP due to various hetero- or homozygous mutations in the human liver/bone/kidney alkaline phosphatase (ALPL) gene [9, 14]. HPP is a rare inherited

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disorder of bone and mineral metabolism with an estimated prevalence of 1:300,000 for the perinatal/infantile HPP and a frequency of 1:6,300 for heterozygous pathogenic variants in the ALPL gene in Europe [15]. The clinical manifestations of HPP are heterogeneous and may include fractures after minor trauma, pseudofractures, gait disturbance, muscle weakness and bone pain in adults, premature tooth loss, insufficient mineralisation of bones and delayed motor development in childhood [9, 14]. The infantile manifestation of the disease has a high mortality rate [16].

The ALPHA study (**A**nalysis of the signs and symptoms related to **L**ow alkaline **PH**osphatase in children and **A**dolescents) was conducted to characterise the clinical presentation of children and adolescents with repeated readings of low ALP activity. A broad awareness of the relevance of low ALP activity as an important biochemical parameter is a fundamental element for early diagnosis of patients with the rare disorder HPP often presenting with unspecific manifestations.

Methods

Study design and sampling frame

The non-interventional retrospective ALPHA study was sponsored by the pharmaceutical company Alexion Pharma Germany GmbH. To obtain a sufficiently large set of data from children presenting with persistently low ALP levels in paediatric clinics in Germany a multicentre approach was adopted. This cross-sectional study collected data from patient laboratory and medical records over six months in 2016–2017 at nine German specialised centres (Supplementary Table 1A). Laboratory records were screened to identify patients with repeated readings of low ALP activity over five years prior to the start of the study. Subsequently, a patient chart review was performed. The number of datasets to be included per centre was restricted to a maximum of 100 with no minimum number of records per centre (Supplementary Table 1B).

Records for patients <18 years of age at the time of abnormal ALP value detection with ALP readings below the lower reference limit were eligible. ALP enzyme activity was defined as low based on age-and sex-adjusted local standards. If none was available, a cut-off value of 100 U/L until the age of 12 years for girls and 14 years for boys and in older adolescents of 50 U/L for girls and 70 U/L for boys were used. Thereby, a safety margin with respect to the reference range was introduced, to ensure that all included ALP readings were below the established lower limit of normal [8, 10, 17]. Data were excluded if patients (1) had at least one ALP assessment within the age- and sex-adjusted normal range by history, (2) had a diagnosis of an oncological or a haematological disorder or (3) were in need of intensive care at the time of ALP assessment.

Data collection

Demographic data, biochemical data and clinical data, such as diagnosis, signs and symptoms and information about medication at the time of low ALP assessment, were collected. The data were entered at the centre into an electronic database, without links to any personal subject identifiers like name or date of birth. To ensure anonymization, a randomised centre ID was allocated to each participating site by an independent third party, so that neither the sponsor nor the evaluators could match a dataset to an individual patient or to a participating site. According to applicable German law, a scientific analysis based on a mere collection of anonymised data from routine clinical records does not require approval by a review board. Approval of the institutional ethics committees was therefore waived, and informed consent was not required.

The following variables were extracted from patient records and entered into a GCP-compliant electronic data capture system (MARVIN, xclinical, Munich, Germany):

- *Demographic data:* Age (years), sex (male, female), height (cm) and weight (kg) at the time of the most recent ALP value.
- *Biochemical data*: Most recent serum ALP values in U/L. Calcium and phosphate serum levels are classified as “normal”, “low” or “high”.
- *HPP:* A diagnosis of HPP at the time of the most recent ALP measurement was indicated as “yes” or “no”. A diagnosis of HPP was established according to the routine procedure of the respective centres. No specific diagnostic criteria were defined per study protocol.
- *Other diagnoses and medications*: Other diagnoses and medications could be entered as free text.
- *Signs and symptoms:* Presence of the following symptoms typical for HPP was specified: short stature, muscle weakness, delayed motor development, gait abnormalities, bone deformities, hypomineralisation, seizures, muscle/bone/joint/lower extremity pain, respiratory disorder, high phosphate or high calcium. All were assessed as “yes” or “no”.

Data evaluation

To adjust for sex and age, z-scores for height, weight and ALP values were determined [10, 18]. ALP z-scores were calculated using ALP percentile reference data from Zierk et al. [10]. Percentiles of observed ALP values were calculated (with interpolation) using age and gender-matched percentile reference data, and z-scores were calculated based on the determined ALP percentiles for observed ALP values. ALP level of zero was used as zero percentile for all ages to calculate percentiles of ALP values that are below the 1st percentile. For the calculation of height and weight z-scores (Z), the formula for a given physical measurement X (weight or length) is: $Z = \frac{\left(\left(\frac{X}{M}\right)^{L}\right)-1}{LS}, L \neq 0$ or $Z = \frac{\ln\left(\frac{X}{M}\right)}{S}, L = 0$, where L, M and S are the estimated parameters from the CDC growth chart reference data corresponding to the age [18]. Height and weight z-scores above 4 or below −4 were assessed for plausibility, and non-plausible values were excluded.

For further analyses, data sets of the total cohort (Group 1) and the following subgroups were considered: data sets with diagnoses other than HPP or unclear diagnoses (Group 2), and datasets with a documented diagnosis of HPP (Group 3). Incidences of signs and symptoms typical for HPP and the number of concomitant typical symptoms per patient were calculated in the subgroups of patients without (Group 2) and with (Group 3) documented HPP.

All diagnoses other than HPP as well as medications that were entered into the database were screened for the presence of one or more known conditions associated with low serum ALP values. The

following conditions were considered relevant: hypothyroidism, calorie depletion, zinc deficiency, magnesium deficiency, vitamin B12, C or D deficiency, previous major surgery or trauma, severe anaemia, celiac disease, growth hormone deficiency, impaired kidney or liver function, Wilson's disease, abnormal serum parathyroid hormone or ceruloplasmin levels, as well as the administration of contraceptives/oestrogen or steroids [2, 19–26]. If multiple entries were documented for a single patient, all were considered. The respective incidences were calculated for the total cohort.

To assess whether there is a correlation between low serum ALP levels and body height, a potential relationship was evaluated using calculated z-scores for both variables.

Medians and interquartile ranges (IQRs), as well as 25th and 75th percentiles, were calculated for continuous variables. Proportions were determined for categorical parameters. Linear regression to study the relationship between z-scores of body height and ALP values was calculated based on the Least Squares method. A two-sided F-test with a cut-off value for significance of $p<0.5$ was applied. The analyses were done using R software (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria).

Bias was reduced by using a multicentre approach and a random sampling of records provided by the laboratories, including multiple TNSLAP readings when available. As the study objectives were explorative, formal sample size estimation was not required.

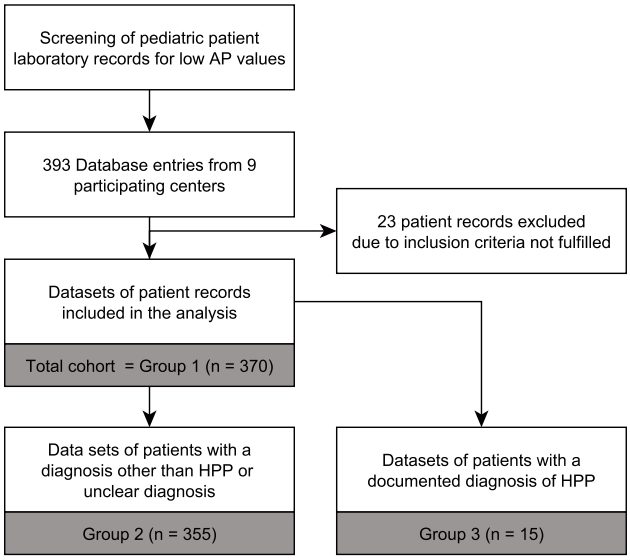

Figure 1: Flowchart depicting disposition of patient database entries and assignment to the analysis groups.

Results

Study cohort

Data for 393 patients from the nine participating centres were entered into the database. As data were fully anonymised, the patient IDs could not be allocated to the respective centres but only to the centre ID. Records of 23 patients were excluded from the data set as per the exclusion criteria, resulting in 370 data sets for further analysis (Figure 1). Additionally, three and nine patients were excluded from the evaluation related to height and weight based on non-plausible z-score values of above 4 or below −4, respectively. Missing data for specific variables resulted in a smaller group size available for the respective analysis.

Out of 370 patients (Group 1), 15 patients had a confirmed diagnosis of HPP (Group 3), whereas the subgroup without a diagnosis of HPP (Group 2) comprised 355 patients. Due to the strongly inhomogeneous sample sizes and patient age distribution of the subgroups, no inferential statistics were deemed feasible. Therefore, only the results of the descriptive statistics are presented. The demographic data are summarised in Table 1.

ALP values

The median ALP activity in the total cohort was 62.0 U/L (IQR = 42.0 U/L) and the respective z-score was −2.07 (IQR = 0.87). The ALP serum levels were on average lower in the group with established HPP diagnoses (Group 3) compared to the Group 2 with medians of 52.0 U/L (IQR = 44.0 U/L) vs. 64.0 U/L (IQR = 47.9 U/L). The median ALP z-score values were −2.52 (IQR = 0.20) and −1.96 (IQR = 0.87), accordingly.

Diagnoses and medications associated with low ALP levels in the core group

For 241 (67.9%) patients without a confirmed HPP diagnosis (Group 2), one or more medical conditions were specified, which are known to be associated with low ALP serum levels other than HPP. Table 2 provides the respective incidences. In the data sets of 54 (15.2%) patients, the diagnosis was stated as not completed. For the remaining 60 (16.9%) patients there was insufficient information to allocate them to the listed specific conditions.

Signs and symptoms

The pattern of signs and symptoms overlapped between the core group and the group with documented HPP, and patients with clinical features consistent with HPP could be found in both groups. As expected, relevant symptoms were relatively more frequent and more clustered in the HPP group. Six of the HPP patients had two or more

Table 1: Demographic parameters of the total cohort (Group 1), patients without (Group 2) and the group of patients with documented HPP (Group 3).

	Male, n, %	Female, n, %	Median age in years [IQR]	Median height in cm [IQR]	Height z-score [IQR]	Median weight in kg [IQR]	Weight z-score [IQR]
Group 1 (total cohort)	140 [38%]	230 [62%]	14.33 [6.56–16.67] (n=370)	155.0 [110.0–167.8] (n=342)	−0.23 [−1.42–0.91] (n=342)	49.0 [23.0–63.0] (n=326)	0.07 [−1.10–1.04] (n=326)
Group 2 (patients without documented HPP)	137 [39%]	218 [61%]	14.92 [8.88–16.83] (n=355)	157.0 [121.0–169.0] (n=328)	−0.17 [−1.74–1.42] (n=328)	49.9 [24.2–63.3] (n=311)	0.05 [−1.10–1.00] (n=311)
Group 3 (patients with documented HPP)	3 [20%]	12 [80%]	3.67 [1.54–9.88] (n=15)	94.0 [84.0–130.0] (n=14)	−1.03 [−1.62–0.05] (n=14)	13.9 [11.5–27.8] (n=15)	−0.35 [−1.43 to −0.08] (n=15)

Table 2: List of clinical conditions associated with low ALP serum levels other than HPP and incidence in Group 2.

Clinical conditions associated with low AP values other than HPP reported as	Incidence
Hypothyroidism	44 (11.9%)
Administration of steroids	41 (11.1%)
Calorie depletion (anorexia, malnutrition)	41 (11.1%)
Administration of contraceptives/oestrogen	34 (9.2%)
Severe illness	29 (7.8%)
Abnormal renal or liver function	26 (7.0%)
Vitamin B12, C or D deficiency	26 (7.0%)
Growth hormone deficiency	10 (2.7%)
Anaemia	8 (2.2%)
Celiac disease	3 (0.8%)
Magnesium deficiency	2 (0.5%)
Abnormal parathyroid hormone levels	1 (0.3%)
Previous major surgery or trauma	–
Achondroplasia	–
Wilson's disease	–
Zinc deficiency	–
Abnormal serum ceruloplasmin levels	–

characteristic symptoms (Figure 2A). Short stature was the most prevalent feature in both subgroups (Figure 2B).

Relationship between body height and ALP levels

Interestingly, a weak statistically significant positive linear relationship between z-scores of ALP levels and the body height was identified (Figure 3):

$$\text{Height}_{\text{z-score}} = 0.88 * \text{ALP} - \text{value}_{\text{z-score}} + 1.52, R^2 = 0.09,$$
$$p = 5.83e - 8.$$

Discussion

The ALPHA study collected retrospective data from laboratory and patient medical records for a relatively large group of paediatric patients with low ALP values from nine specialised centres throughout Germany. Most interestingly, a weak positive correlation between age- and sex-adjusted ALP activity and body height was detected. Twelve medical conditions associated with low ALP activity other than HPP were identified, whereby only 15 out of the 370 records included in the analysis had a documented diagnosis of HPP. Signs and symptoms characteristic of HPP could be found in the entire cohort and, as expected, were more frequent in patients with HPP.

Not all aspects with regard to the physiological role of ALP are fully understood, and there are still open questions regarding potential sequelae of low serum ALP activity [23]. Some have been recently addressed. Lopez-Delgado et al. suggested that individuals with persistently low serum ALP levels have an overall reduction of bone turnover [21]. This observation was made in the absence of overt clinical symptoms and rated as resembling subclinical abnormalities of bone remodelling or bone mass. In a recent publication on bone turnover markers in healthy prepubertal children, Monjardino et al. could demonstrate a weak positive linear relationship between body height and serum ALP levels [27]. This is in line with the results of the ALPHA study, and a correlation may be present within normal as well as within low ALP activity ranges. A relationship between ALP z-scores and body height found in this cohort further substantiates the relevance of low ALP values as one of the potential markers of disturbed bone metabolism.

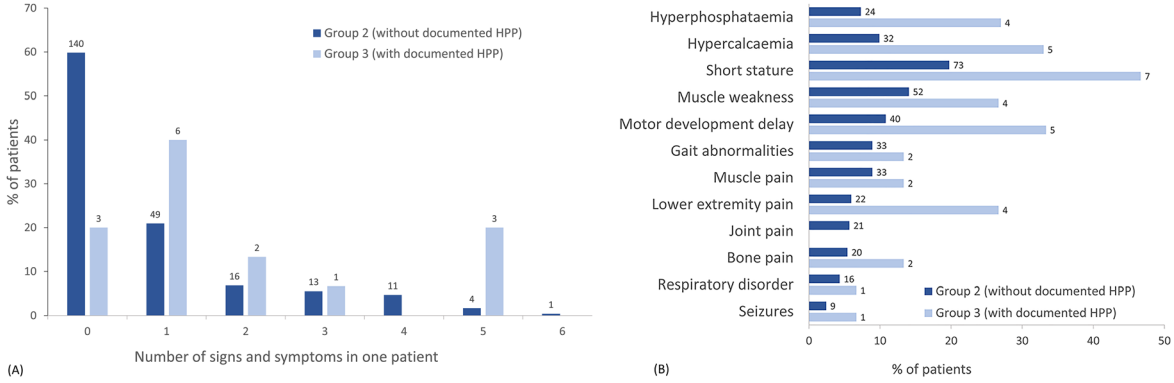

Figure 2: (A) Accumulation of typical signs and symptoms in individual patients. (B) Percentage of patients presenting with symptoms, which are typically associated with HPP in the group of patients without (Group 2) and with documented HPP diagnosis (Group 3).

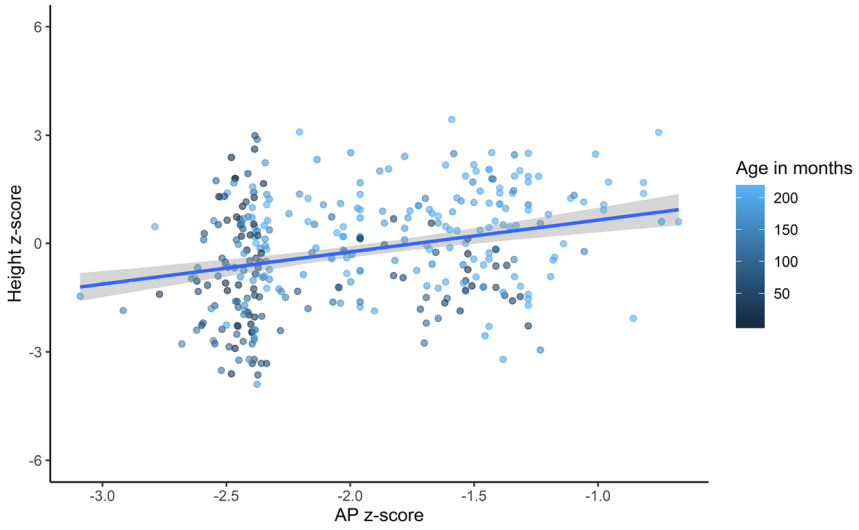

Figure 3: Relationship between body height and ALP values (as z-scores) in the total cohort (Group 1; p=5.83e-8).

Deficient bone mineralisation is characteristic for patients with HPP. The typical manifestations of HPP, including premature loss of deciduous teeth, musculoskeletal pain, recurrent poorly healing fractures and mobility restrictions, have a substantial impact on health-related quality of life [28, 29]. However, due to the rarity of the disorder, unspecific presentation in milder forms of the disease and lack of awareness among physicians, significant diagnostic delay or even missed diagnosis is common. Median delay to diagnosis has been shown to be more than one year in children and about 10 years in adults [30]. A diagnosis of HPP should be considered based on typical signs and symptoms in combination with repeatedly low ALP serum activity, which cannot be explained otherwise [11, 20, 24].

The majority of the patients in the present study had causes other than HPP underlying low ALP activity, and only 4.1% had an established diagnosis of HPP. Also, the symptoms largely overlapped between the documented HPP cases and the subgroup without a diagnosis of HPP, highlighting, that low ALP activity or HPP-related symptoms alone are common, but unspecific parameters. Further diagnostic follow-up in cases of unexplained low ALP levels is recommended.

Study limitations

The main limitation of the study is its retrospective character. As it was cross-sectional data analysis, no causal relationships can be deduced. Therefore the origin of the low ALP readings in many patients remains unclear. In addition, data quality is heterogeneous, since the information provided from the patient records was partially incomplete and the diagnostic procedure used for establishing the HPP diagnosis was not assessed. Further, it was not part of the study to follow-up the patients based on their records. Therefore, it is possible that within the group of low ALP readings, cases of undiagnosed HPP may be included, which would result in a partial overlap of the subgroups. While the sampling frame was not rigorously defined, we consider a random selection from available laboratory records at nine specialised centres throughout Germany a valid database. Bias was reduced by clearly predefined in- and exclusion criteria, a GCP-compliant data management and a multicentre approach in an academic setting.

Conclusions

In this data analysis based on patient medical records of a paediatric cohort with repeatedly low ALP values, we found a broad spectrum of underlying disorders with known effects on ALP readings. Of interest is the confirmed association of TNSALP levels and height in children and adolescents, which should be further investigated in a longitudinal cohort setting. Persistently low ALP values accompanied by specific symptoms should raise suspicion of HPP as a differential diagnosis.

Acknowledgements: We wish to thank the investigators, Georg Christof Schwabe, Children's Hospital, Carl-Thiem-Klinikum, Cottbus, Volker Böttcher and Janu Anton, Endokrinologikum, Frankfurt am Main, Beatrix Schmidt, St. Joseph's Hospital, Berlin, Dorothee Schmidt, Department of Pediatrics, University of Lübeck, Lübeck, Michael

Wallot, Department of Pediatrics, Bethanien Hospital, Moers and staff from all sites participating in this study. The authors thank Susanne Merk for providing medical writing and data analysis support. We are grateful to Roland Derwand and Alexandra Aliluev, of Alexion Pharma Germany GmbH (Alexion), for support in the review of the manuscript.

Research funding: This study was sponsored by Alexion Pharma Germany GmbH. Funding was also provided for a medical writing support to Susanne Merk, SUME Medical Writing.

Author contributions: Study concept and design: CG and AP. Acquisition of data: CG, OS, CJP, AMD and study site investigators. All authors who contributed to data interpretation, writing and review of the manuscript have accepted the responsibility for the entire content of this submitted manuscript and approved submission.

Competing interests: CG, OS, CJP and AMD are clinical study investigators in the ALPHA study. OS obtained research funding, received honoraria and travel support from Alexion for consulting and participation on advisory boards, etc. within the past three years. AMD received honoraria from Alexion for scientific lectures and cosponsoring of a "Hands-on Workshop on lysosomal storage diseases". CG received honoraria from Alexion for participation in advisory boards. AP is an employee of Alexion, the study sponsor and may have stock options. The funding organization was involved in the study design, analysis and interpretation of data and in the writing of the publication. The decision to submit the manuscript was taken by mutual agreement between the funding organisation and the authors.

Informed consent: Informed consent was not required.

Ethical approval: According to applicable German law, a scientific analysis based on a mere collection of anonymised data from routine clinical records does not require approval by a review board. Approval of the institutional ethics committees was therefore waived.

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/jpem-2021-0330).