European Journal of Medical Genetics 66 (2023) 104866

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Serum alkaline phosphatase can be elevated in patients with hypophosphatasia due to liver disease

Evert F.S. van Velsen [a,b,*], Zografia Zervou [a,b], M. Carola Zillikens [a,b]

[a] Department of Internal Medicine, Erasmus Medical Center, University Medical Center, Dr. Molewaterplein 40, 3015 CE, Rotterdam, the Netherlands
[b] Erasmus MC Bone Center, Erasmus Medical Center, University Medical Center, Dr. Molewaterplein 40, 3015 CE, Rotterdam, the Netherlands

ARTICLE INFO

Handling Editor: A. Verloes

Keywords:
Hypophosphatasia
Alkaline phosphatase
TNSALP
ALP

ABSTRACT

Background: Hypophosphatasia (HPP) is a rare inherited disorder caused by pathogenic loss-of-function variants in the *ALPL* gene, encoding the tissue-nonspecific isoenzyme of alkaline phosphatase (ALP; TNSALP). Low serum ALP is the biochemical hallmark of HPP, but it is unknown whether ALP levels can increase due to concurring liver disease, which may lead to a missed diagnose of HPP. We present a patient with genetically confirmed HPP, who showed a transient increase of serum ALP levels due to alcohol-induced hepatitis.

Clinical report: A 71-year old man was seen at our Bone Center for surveillance of HPP. Serum ALP was always low (23 U/L; reference value: <115 U/L). During follow-up, his serum ALP increased (156 U/L, further rising to 204 U/L), with concomitantly elevated serum gamma-glutamyl transferase and transaminases, and a rise in bone specific ALP (18.7 μg/L; reference value: 5.7–32.9 μg/L). This was attributed to alcohol-induced hepatitis. After refraining from alcohol intake, both serum ALP and bone specific ALP levels returned to initial low levels (30 U/L and 4.3 μg/L respectively).

Conclusions: We demonstrated the history of a 71-year old patient with HPP, presenting during routine follow-up with an elevated serum ALP level up to 204 U/L due to alcohol-induced hepatitis. This case illustrates that the diagnosis of HPP can potentially be missed when ALP levels are normal or elevated due to a concomitant liver disease.

1. Introduction

Hypophosphatasia (HPP) is a rare, heterogeneous, inherited disorder of bone and mineral metabolism caused by pathogenic loss-of-function variants in the *ALPL* gene, encoding the tissue-nonspecific isoenzyme of alkaline phosphatase (ALP; TNSALP) (Bianchi et al., 2020); more than 400 different pathogenic variants have been described. Four distinct genes encode distinct ALP isoforms, namely intestinal, placental, germ-cell, and the non-specific ALP (TNSALP) (Makris et al., 2023). TNSALP forms the main fraction of total serum ALP, and therefore increased serum ALP levels are usually due to increased TNSALP, which is predominantly expressed in the bone and liver. Due to post-translational modifications, the carbohydrate composition of bone and liver ALP is distinct. TNSALP hydrolyzes pyrophosphate to provide inorganic phosphate necessary to promote mineralization (Makris et al., 2023). Elevated levels of pyrophosphate impair osteoid mineralization, and promote extracellular mineral accumulation, which may lead to chondrocalcinosis and deposition of calcium in periarticular structures (Riancho, 2023; Guanabens et al., 2014).

The clinical manifestations of HPP are highly variable, depending on the age of onset, the type of genetic variant, and inheritance (autosomal recessive or dominant). Several forms are described based on the age of manifestation (perinatal, prenatal, infantile, childhood, and adult). The most severe forms are those affecting infants and young children, while some adults hardly have any complaints (Bianchi et al., 2020). The main clinical signs are related to defective bone and tooth mineralization (rickets in children, osteomalacia, fractures, dental problems), but other systemic manifestations (e.g., seizures, respiratory and kidney problems, chronic musculoskeletal pain, muscle weakness) may be present (Makris et al., 2023). Low serum ALP is the biochemical hallmark of HPP and therefore, in patients with normal or elevated serum ALP, the diagnosis of HPP is usually not considered, despite a clinical suspicion (Riancho, 2023; Schmidt et al., 2021). Additional diagnostics include serum pyridoxal 5′-phosphate (PLP; active form of vitamin B6) and urine

* Corresponding author. Erasmus MC Bone Center, Department of Internal Medicine, Erasmus Medical Center, University Medical Center, Dr. Molewaterplein 40, 3015 CE, Rotterdam, the Netherlands.
E-mail addresses: e.vanvelsen@erasmusmc.nl (E.F.S. van Velsen), z.zervou@erasmusmc.nl (Z. Zervou), m.c.zillikens@erasmusmc.nl (M.C. Zillikens).

https://doi.org/10.1016/j.ejmg.2023.104866
Received 23 June 2023; Received in revised form 16 August 2023; Accepted 1 October 2023
Available online 13 October 2023

phospho-ethanolamine levels, which are typically both increased (Bianchi et al., 2020). Although a life-long low serum ALP is one the biochemical hallmarks of the disease, it is not well-known whether, and to what extent, ALP levels can increase in HPP during a concomitant liver disease. Here we present a 71-year old man with genetically confirmed HPP, showing a transient elevation of serum ALP levels due to alcohol-induced hepatitis.

2. Clinical Report

A 71-year old man was seen at the Erasmus MC Bone Center for surveillance of his known HPP. He was diagnosed two years earlier when he presented with a burning sensation in his left arm and left leg which was diagnosed as polyneuropathy. Additionally, subtle calcifications at the insertion of his right supraspinatus tendon were seen on X-ray. His dental history revealed the extraction of several teeth, starting at the age of 18 years. Laboratory examination showed a low serum ALP (23 U/L; reference value: <115 U/L; Roche COBAS, Roche, Mannheim, Germany) and a high serum vitamin B6 (390 nmol/L; reference value: 35–110 nmol/L; UHPLC-MS/MS, in house developed assay). Additional genetic testing revealed a heterozygous variant of the *ALPL* gene (NM_000478.5 (ALPL):c.331G > A, p. (Ala111 Thr), fitting with the clinical diagnosis of adult onset HPP.

During follow-up, his serum ALP was stable, but at a regular visit two years later, serum ALP was 156 U/L, with an increase in bone specific ALP level compared to previous values (18.7 μg/L; reference value: 5.7–32.9 μg/L; IDS-ISYS, Immunodiagnostic Systems, Boldon, UK). A concomitant increase of other liver enzymes was seen (see also Table 1). Extensive additional analyses showed no signs of an infectious (Hepatitis A, B, C, D and E, Epstein-Barr virus, Cytomegalovirus and Parvo B19 virus) or immunologic cause, while liver ultrasound showed a normal gallbladder, bile ducts, liver vessels and liver parenchyma; there were no signs of cirrhosis or steatosis. Medical history revealed a (self-reported) alcohol consumption of ten bottles of beer per week (alcohol percentage 5–15%), which we advised him to stop. Maximum levels of serum ALP and gamma-glutamyltransferase (GGT) were 204 U/L and 486 U/L respectively. After six months, the serum ALP was back at the usual level (33 U/L; see Fig. 1), as was the bone specific ALP level (4.3 μg/L). Additionally, also GGT normalized (21 U/L; reference value: <55 U/L). Therefore, we advised the patient to keep refraining from drinking alcohol, and 5 months later, almost one year after the initial rise, the serum ALP remained stably low (30 U/L).

3. Discussion

HPP is a very rare metabolic bone disease due to shortage of ALP with a large variety in type and severity of clinical manifestations. One of the diagnostic hallmarks of HPP is a decreased serum ALP level, which may point towards the diagnosis in relative mild disease (Riancho, 2023). At our bone expertise center we wondered for some time whether, and to what extent, serum ALP can increase during concurring diseases, such as liver disease (e.g., hepatitis, cirrhosis, or bile duct obstruction), as this could potentially result in a missed diagnosis of HPP. However, we could not find any relevant publications on this topic. In theory, as HPP is caused by a pathogenic variant in the *TNSALP* gene, which is predominantly expressed in bone and liver, one might expect that serum ALP would remain low during liver disease. Recently, we saw this 71-year old patient with adult HPP due to a heterozygous variant of the *ALPL* gene (NM_000478.5 (ALPL):c.331G > A, p. (Ala111 Thr), who presented during routine follow-up with an elevated serum ALP level up to 204 U/L, which we contributed to an alcohol-induced hepatitis. After refraining from alcohol intake, both serum ALP and bone specific ALP levels returned to low initial levels (30 U/L and 4.3 μg/L respectively). This case illustrates that serum ALP levels can increase during concurring liver disease. Possible explanations are that due to injury of liver cells, more ALP is released into the circulation, resulting in increased serum levels of serum ALP (Torkadi et al., 2014), or that, as the patient has a heterozygous variant of the *ALPL* gene, the normal gene was able to increase the TNSALP serum levels. Our patient showed a temporary increase of serum ALP levels concomitant with increased liver enzymes. Also serum bone specific ALP rose, probably due to the known cross reactivity of the bone and liver isoforms, which is <9% according to the manufacturer of the assay our laboratory uses, but it can be as high as 18% according to literature for the different used assays (Makris et al., 2023; Brady et al., 2019). This difficulty of separating bone and liver isoforms is mainly caused by that fact that they share the same amino acid sequence and that their differences are limited only to the rate of post-translational glycosylations (Makris et al., 2023). Therefore, when interpreting bone specific ALP one should always take total serum ALP and liver enzymes into account. Combined with the increased liver enzymes, it is highly likely that the increase in serum ALP in our patient

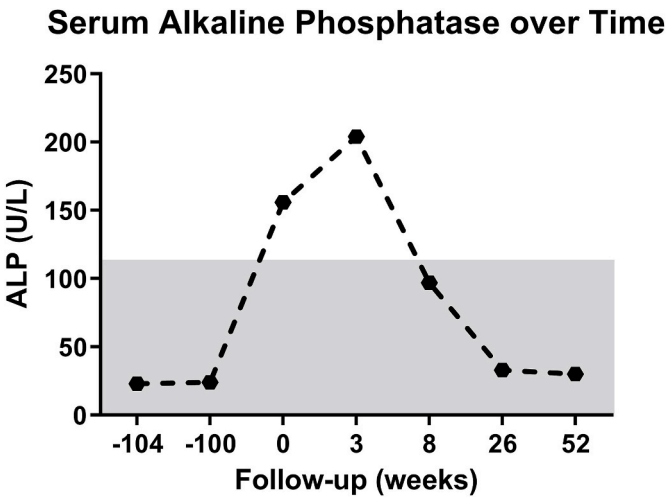

Fig. 1. Serum Alkaline Phosphatase (ALP) over Time.
Caption: the shaded area shows the reference interval (<115 U/L).

Table 1
Laboratory values over time.

	Reference Range	Units	At Diagnosis	At Presentation	After 3 weeks	After 6 months	After 12 months
Serum							
Calcium	2.20–2.65	mmol/L	2.42	2.43	2.37	2.47	2.38
Phosphate	0.80–1.40	mmol/L	–	1.49	1.18	1.31	1.37
Albumin	35–50	g/L	44	36	35	41	38
Creatinin	50–100	umol/L	98	112	100	94	97
Alkaline Phosphatase	<115	U/L	23	156	204	33	30
Bone Alkaline Phosphatase	5.7–32.9	μg/L	–	–	18.7	5.3	4.3
Gamma-glutamyltransferase	<55	U/L	16	452	486	28	21
Aspartate aminotransferase	<35	U/L	23	–	131	–	34
Alanine transaminase	<45	U/L	21	–	163	–	25
Vitamin B6	35–110	nmol/L	390	–	–	–	–

was caused by the liver fraction. The increase in liver enzymes was most likely due to alcohol-induced hepatitis, even though the alcohol consumption of ten bottles of beer per week (alcohol percentage 5–15%) was not that high. However, self-reported alcohol intake is prone to underestimation (Devaux and Sassi, 2016). To further support the diagnosis of alcohol-induced hepatitis, we ruled out several other causes of hepatitis, and liver enzymes normalized after stopping the alcohol consumption.

Our case demonstrates that a parenchymal liver disease may lead to increased serum ALP levels in patients with (heterozygous forms of) HPP, which could camouflage the disease. This may not only result in a missed diagnosis and lack of explanation for the symptoms of the patient, but also in a potential inappropriate treatment with bisphosphonates in case of fractures, which may exacerbate the bone phenotype in HPP (Rassie et al., 2019). Limitations to our report include the cross-reactivity of the assay for measuring serum bone ALP between bone and liver isoforms, which probably have led to a falsified increased bone ALP. Finally, as in many hospitals, our laboratory unfortunately does not indicate a lower limit for serum ALP. However, a level of below 30 U/L, like in our patient, is usually indicated as being too low. Having a lower limit for serum ALP is important for clinicians, especially for those who do not often see patients with HPP, to become aware of this potential diagnosis, or to evaluate other causes for low ALP (Riancho, 2023; Schmidt et al., 2021).

In conclusion, this case of a patient with genetically confirmed adult onset HPP shows that serum ALP levels can increase during (alcohol-induced) hepatitis, which may potentially lead to a missed diagnosis of HPP. This reinforces the notion that in case of even a mild suspicion of HPP, serum ALP levels should be interpreted in relation to liver enzymes before HPP can be ruled out.

Author disclosure statement

EVV, ZZ and MCZ declare no conflicts of interest and no competing financial interests exist. We did not use artificial intelligence (AI) and AI-assisted technologies in the writing process.

Funding statement

This publication has been supported by ERN BOND - European Reference Network for rare BONeDiseases, which is partly co-funded by the European Union within the framework of the EU4Health Programme 2021–2027."

4. Patient consent statement

The described patient gave written informed consent for publication of this manuscript.

CRediT authorship contribution statement

Evert F.S. van Velsen: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Zografia Zervou:** were involved in, analysis, and treatment of the patient. **M. Carola Zillikens:** were involved in, Formal analysis, and treatment of the patient, All authors reviewed and revised the manuscript to improve its intellectual and technical content, Conceptualization, Writing – review & editing.

Data availability

The data that has been used is confidential.

References

Bianchi, M.L., Bishop, N.J., Guanabens, N., Hofmann, C., Jakob, F., Roux, C., et al., 2020. Hypophosphatasia in adolescents and adults: overview of diagnosis and treatment. Osteoporos. Int. 31 (8), 1445–1460. Epub 2020/03/13.

Brady, J.J., McGoldrick, D., O'Callaghan, K., McNamara, F., Mulready, K.J., Cullen, M.R., et al., 2019. Bone alkaline phosphatase on the IDS-iSYS automated analyser; cross-reactivity with intestinal ALP. Clin. Chem. Lab. Med. 57 (8), e186–e188. Epub 2018/12/12.

Devaux, M., Sassi, F., 2016. Social disparities in hazardous alcohol use: self-report bias may lead to incorrect estimates. Eur. J. Publ. Health 26 (1), 129–134. Epub 2015/11/21.

Guanabens, N., Mumm, S., Moller, I., Gonzalez-Roca, E., Peris, P., Demertzis, J.L., et al., 2014. Calcific periarthritis as the only clinical manifestation of hypophosphatasia in middle-aged sisters. J. Bone Miner. Res. 29 (4), 929–934. Epub 2013/10/15.

Makris, K., Mousa, C., Cavalier, E., 2023. Alkaline phosphatases: biochemistry, functions, and measurement. Calcif. Tissue Int. 112 (2), 233–242. Epub 2022/12/27.

Rassie, K., Dray, M., Michigami, T., Cundy, T., 2019. Bisphosphonate use and fractures in adults with hypophosphatasia. JBMR Plus 3 (10), e10223. Epub 2019/11/07.

Riancho, J.A., 2023. Diagnostic approach to patients with low serum alkaline phosphatase. Calcif. Tissue Int. 112 (3), 289–296. Epub 2022/11/09.

Schmidt, T., Schmidt, C., Amling, M., Kramer, J., Barvencik, F., 2021. Prevalence of low alkaline phosphatase activity in laboratory assessment: is hypophosphatasia an underdiagnosed disease? Orphanet J. Rare Dis. 16 (1), 452. Epub 2021/10/30.

Torkadi, P.P., Apte, I.C., Bhute, A.K., 2014. Biochemical evaluation of patients of alcoholic liver disease and non-alcoholic liver disease. Indian J. Clin. Biochem. 29 (1), 79–83. Epub 2014/01/31.