Unusually low serum alkaline phosphatase activity in a patient with acute on chronic liver failure and hemolysis

Parul Arora¹, Shekhar Singh Jadaun², Prasenjit Das³, Shalimar², Sudip K. Datta¹

¹Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India
²Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, India
³Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

INFO

Corresponding author:
Sudip Kumar Datta
Department of Laboratory Medicine
All India Institute of Medical Sciences
New Delhi, 110029
India
Phone: 011-26594629
Cell: 9868397278; 9350179698
E-mail: drsudipdatta@gmail.com

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CASE REPORT

A 28-year-old male with acute on chronic liver failure (ACLF) and hepatic encephalopathy had deranged liver function with curiously low level (0-15 IU/L) of serum alkaline phosphatase (ALP). Peripheral smear examination suggested hemolytic anemia. The finding of persistent low ALP, after ruling out pre-analytical causes, in ACLF has been reported in Wilson’s disease (WD) with/ without autoimmune hemolytic anemia (AIHA). Definitive evidences of WD were not seen in our case. Positive DCT and histological features suggest a diagnosis of autoimmune hepatitis with secondary hemochromatosis and cholangitis. Low ALP might not always be a determinant of bile duct pathology in patients of ACLF with AIHA.
INTRODUCTION

Acute on chronic liver failure (ACLF) is acute deterioration of liver function in patients with pre-existing liver disease. The American Association for the Study of Liver Diseases defined ACLF as: “acute deterioration of pre-existing chronic liver disease usually related to a precipitating event and associated with increased mortality at 3 months due to multi-system organ failure” (1). Common causes of ACLF include active alcohol consumption, reactivation of hepatitis B virus infection, superinfection with hepatitis E virus, autoimmune hepatitis flare, sepsis and superimposed drug or toxic injury.

In our case the patient presented with acute liver failure and hemolytic anemia. However, he was detected with chronic liver disease (CLD) two years back. Most causes of ACLF were ruled out. However, an unusual finding of low serum alkaline phosphatase (ALP) values (<5-15 IU/L) was observed. Possible causes for the same in the setting of ACLF were looked for.

CLINICAL – DIAGNOSTIC CASE

A 28-year-old man presented to the emergency with jaundice for two weeks and progressively increasing abdominal distension. Two days prior to admission he developed malena, decreased urine output and drowsiness. His past records revealed that he was diagnosed with CLD, however the etiology was unknown. He recovered spontaneously during that episode and was asymptomatic till the present admission. His relatives denied any history of blood transfusion, intravenous drug abuse, tattooing, promiscuity, consumption of alcohol or smoking.

On assessment in casualty, the patient was found to be icteric and hypotensive. His abdomen was distended; without any guarding or rigidity. Neurological examination suggested the presence of grade III hepatic encephalopathy. Kayser–Fleischer ring was not visualized on slit lamp microscopy. Chest and cardiovascular examination was within normal limits.

On admission he was suspected to be a case of ACLF, which was confirmed in the subsequent two days. His blood investigations revealed: blood urea nitrogen (BUN): 59 mg/dL, serum creatinine: 3 mg/dL, serum total bilirubin: 24.1 mg/dL, direct bilirubin: 17.8 mg/dL, aspartate aminotransferase (AST) levels: 205 IU/L, alanine aminotransferase (ALT): 23 IU/L and ALP: 43 IU/L. Blood ammonia level was elevated to 135 µmol/L. Details of the serial liver function are shown in Table 1. It was observed that serum ALP values decreased to very low (5-15 IU/L) to even undetectable levels over the next few days.

Prothrombin time, INR and activated partial thromboplastin time were prolonged (Table 1). Hemoglobin and platelet counts were low- 6.4 g/dL and 50,000/mm³ respectively. Leucocytes were normal in count and morphology. Peripheral smear examination was suggestive of hemolytic anemia. Direct Coomb’s test (DCT) was positive and indirect (ICT) was negative.

Serum markers for viral hepatitis (HBsAg, Anti HCV, Total anti-HBcAb, IgM HAV, IgM HEV and HEV RNA) were negative. Autoimmune markers including anti-nuclear antibody, anti-smooth muscle antibody, antibody for liver-kidney microsomal type-1 were negative. Total IgG level was 1590 mg/dL (RI: 840 -1700 mg/dL). Serum copper was 53.5 ug/dL (RI: 70-140 ug/dL) and serum ceruloplasmin 17 mg/dL (RI: 20-60 mg/dL), both marginally on the lower side. 24 hours urine copper estimation could not be done. Urine routine microscopy showed the presence of 12-15 RBC/HPF and 6-8 WBC/HPF. Bile pigments were present in urine. However, urine culture was sterile. His esophagogastroscope showed grade-2 esophageal varices.

The patient was managed with broad-spectrum antibiotics and other supportive measures.
Table 1: Laboratory parameters at admission and follow-up

| Lab parameter                   | Reference interval | 2 months prior to admission | On admission | Day 1 | Day 3 | Day 4 | Day 6 | Day 7 |
|---------------------------------|--------------------|-----------------------------|--------------|-------|-------|-------|-------|-------|
| TLC (x103/μL)                   | 4.0-10.0           | 7.1                         | -            | 11    | 4.9   | 4.5   | 6.3   | 8.1   |
| N-Neutrophils (%)               | -                  | 65                          | 82           | 69    | 75    | 84    | 86    |
| L-Lymphocytes (%)               | -                  | 30                          | -            | 08    | 21    | 10    | 04    | 09    |
| M-Monocytes (%)                 | -                  | 01                          | -            | 08    | 06    | 11    | 07    | 04    |
| E-Eosinophils (%)               | -                  | 03                          | -            | 01    | 03    | 04    | 04    | 01    |
| B-Basophils (%)                 | -                  | 01                          | -            | 01    | 01    | 01    | 00    |
| RBC (x106/μL)                   | 4.5-5.5            | -                           | 1.5          | 1.62  | 2     | 1.9   | 1.13  |
| Hemoglobin (g/dL)               | 13-17              | 7.8                         | -            | 6     | 6.4   | 7.5   | 7.7   | 6.6   |
| HCT (%)                         | 40-50              | 22.4                        | -            | 17    | 17.9  | 21.2  | 22.5  | 15    |
| RDW (%)                         | 11.6-14.0          | 19.8                        | 18.5         | 21.1  | 25.3  | 29.6  | 31.1  |
| Platelet (x103/μL)              | 150-410            | 118                         | 60           | 50    | 80    | 40    | 20    |
| BUN (mg/dL)                     | 7-20               | 20                          | 59           | 38    | 26    | 29    | 54    |
| Creatinine (mg/dL)              | 0.6-1.2            | 1.1                         | 2.4          | 3     | 1.5   | 0.8   | 1     | 2.9   |
| Calcium (mg/dL)                 | 9.2-11.0           | 7.9                         | 8.5          | 9     | 8.3   | 8.7   | 9.4   |
| Phosphate (mg/dL)               | 2.3-4.7            | 3.7                         | 7.1          | 3.4   | 2.5   | 2.8   | 7.3   |
| Uric acid (mg/dL)               | 4-8.5              | 3.3                         | -            | 4.5   | 1.9   | 1.3   | 1.5   | 4.1   |
| Sodium (mEq/L)                  | 136-142            | 120                         | 126          | 132   | 137   | 149   | -     |
| Potassium (mEq/L)               | 3.8-5              | 5                           | 7.6          | 4.3   | 4     | 3.5   | -     |
| Total Bilirubin (mg/dL)         | 0.1-1.2            | 4.8                         | 24.35        | 24.1  | 26.6  | 29.5  | 37.7  | 38.6  |
| Bilirubin conjugated (mg/dL)    | <0.3               | 3.6                         | 17.8         | 19    | 21.7  | 25.7  | 24.6  | -     |
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However, his general condition worsened with progressive jaundice and grade-IV encephalopathy. He later developed shock and died 7 days after admission.

His postmortem liver biopsy showed features of cirrhosis with marked activity [Figure 1A, B, F, & G]. Features of lympho-plasmacytic bile duct injury [Figure 1C & E], interphase hepatitis, features of ascending cholangitis, ballooning of hepatocytes, significant canalicular and intra-cytoplasmic cholestasis [Figure 1D] were present. Significant steatosis or deposition of copper associated protein was not noted with orcein stain. Perl’s prussian blue stain showed features of hemochromatosis, with grade-3 iron deposition in the hepatocyte cytoplasm, Kupffer cells and bile duct epithelial cells [Figure 1H]. Based on the overall features, histological possibility of chronic cryptogenic hepatitis with cholangitis and secondary hemochromatosis were suggested.

DISCUSSION

The index case had three characteristic features: first, hemolytic anemia on peripheral smear with positive DCT; second, hemochromatosis in histopathology; and third, very low to undetectable levels of serum ALP.

Hemolysis in the setting of liver failure can be immune mediated or non–immune mediated. Immune mediated hemolysis is seen in fulminant viral hepatitis, septicemia and autoimmune hemolytic anemia (AIHA). Non-immune mediated mechanisms are implicated in microangiopathic hemolysis - disseminated intravascular coagulation in sepsis, disseminated malignancy, fulminant Wilson’s Disease (WD), viral hepatitis, etc.

In the index case, although serum IgG levels were normal and autoimmune profile was negative; clinical findings, positive DCT and histological features drive towards a possibility of an autoimmune hepatitis with secondary hemochromatosis. Features of ascending cholangitis and marked cholestasis in liver biopsy suggest acute hepatic insult. The gradual rise of AST was not accompanied with a parallel rise in ALT, which points towards a non-hepatic pathology. Due to the presence of hemolytic anemia, the patient had grade-3 iron deposition in the hepatic parenchyma, including the bile duct epithelial cells. AIHA is generally a chronic disorder.

|                                | Total Protein (g/dL) | 6-7.8 | 7 | - | 5.4 | 5.3 | 5.2 | 5.3 | 4.8 |
|--------------------------------|---------------------|-------|---|---|-----|-----|-----|-----|-----|
| Albumin (g/dL)                 |                     | 3.2-4.5 | 2.1 | - | 2.1 | 2   | 2.5 | 2.7 | 2.5 |
| Globulin (g/dL)                |                     | 2.3-3.5 | 4.9 | - | 3.3 | 3.3 | 2.7 | 2.6 | 2.3 |
| AST (IU/L)                     |                     | 8-40   | 87  | - | 68  | 205 | 174 | 88  | 291 |
| ALT (IU/L)                     |                     | 4-45   | 12  | - | 17  | 23  | 17  | 10  | 79  |
| ALP (IU/L)                     |                     | 80-270 | 179 | - | 51  | 43  | Low | Low | Low |
| Arterial blood Ammonia (μmol/L)|                     | 10-35  | -   | - | -   | 121 | 135 | -   | 128 |
| Total IgG (mg/dL)              |                     | 840-1700 | -   | - | -   | -   | -   | -   | 1590 |
which may progress to liver failure. Extrahepatic features of autoimmunity and seroimmunologic changes may be however absent in most cases.

The third interesting finding was a persistently low serum ALP value despite presence of lymphocytic cholangiopathy, ascending cholangitis as well as iron deposition in bile duct epithelial cells. Methodological interferences in ALP assay due to anticoagulant contaminations, commonly with EDTA, were excluded on the basis of other biochemical parameters like calcium and potassium (2). A few cases have been reported in association with WD where serum ALP was undetectable (3,5). A recent report has identified two novel mutations in ATP7B gene which encodes for a membrane-bound copper transporting ATPase in a 42 year woman with WD and low ALP levels (4).

WD is manifested with impaired biliary copper excretion resulting in positive copper balance in liver. Defective copper incorporation in apoceruloplasmin leads to low blood ceruloplasmin levels which in this case was marginally low. Serum copper was also observed to be marginally low. Hepatic decompensation frequently occurs in these patients.

Moreover, in severe liver failure, hemolytic anemia may develop as large amount of copper is released into the circulation due to hepatocellular necrosis. However, KF ring, a pathognomonic feature of WD was not visualized. In our index case, the diagnosis of WD was not considered on histological examination, as there was no macrovesicular steatosis, nuclear glycogenization or evidence of hepatic significant deposition of copper associated protein.

Besides, hypophosphatasia, a rare, genetic disease, characterized by mutations in the tissue non-specific alkaline phosphatase (TNSALP) gene is reported to lead to diminished activity...
of the TNSALP enzyme in target tissues. Clinical features include low levels of ALP in serum and bone, osteomalacia and periodontal disease.

Although, this is commonly described in children, rarely it has also been described in adulthood, when it is most commonly characterized by poor healing, recurrent metatarsal stress fractures, and bone pain. Our index case did not have any of these manifestations and was safely ruled out for the diagnosis of hypophosphatasia. Potential interferences from any drugs in the assay of ALP were also ruled out. Drugs that are reported to interfere physiologically or analytically in ALP measurement include ibuprofen, theophylline, cefoxitin, doxycycline, amphotericin B, tetracycline, antiepileptics, anticoagulants, lipid-lowering drugs, and inhibitors of bone matrix formation and resorption. Our index patient was managed with broad spectrum antibiotics- Piperacillin with Tazobactam. In addition, he also received Rifaximin, Lactulose and other supportive measures. Piperacillin with Tazobactam is not known to cause lowering of ALP levels, rather may sometimes cause transient increase in its levels.

In summary, peripheral blood and positive DCT of the patient indicate a possible presence of AIHA. On histopathology, autoimmune hepatitis with secondary hemochromatosis was suggested. However, ALP is usually raised in cases of hemochromatosis. Hence, in the index case, whether a persistent low ALP level in the setting of hemochromatosis is caused by a sub-clinical WD or AIHA remains debated.

Two cases with WD with superimposed autoimmune features have also been reported, however definitive evidences of WD and AIH were observed in those patients (6).

Therefore, it is important to highlight that, in presence of the above two conditions; the serum alkaline phosphatase may not be a true determinant of any possible bile duct pathology.

LEARNING POINTS

1. Autoimmune hepatitis is an important cause for ACLF and may present without the serological markers for the same.
2. Patients with AIH and WD have been reported and may present with overlapping features of both the diseases.
3. Low ALP, although reported with WD may not always be a pointer towards WD; especially in patients with overlapping features.
4. ALP might not always be a true determinant of bile duct pathology.

Abbreviations:

- ACLF: Acute on Chronic Liver failure
- CLD: Chronic Liver Disease
- ALP: Alkaline phosphatase
- BUN: Blood urea nitrogen
- AST: Aspartate aminotransferase
- ALT: Alanine aminotransferase
- INR: International normalized ratio
- DCT: Direct Coomb’s test
- ICT: Indirect Coomb’s test
- RI: Reference Interval
- AIHA: Autoimmune hemolytic anemia
- WD: Wilson’s Disease

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