False Negative High-Density Lipoprotein-Cholesterol in an Autoimmune Hepatitis Patient: Case Report

Otoimmünün Hepatitli Bir Olguda Yanlış-Negatif Yüksek Yoğunluklu Lipoprotein-Kolesterol

ABSTRACT  False-negative high density lipoprotein-cholesterol (HDL-C) detection is a remarkable condition in clinical laboratory practice. We report an 18-year-old female who admitted to transplantation division, with cirrhotic process of autoimmune hepatitis in which polyclonal hypergammaglobulinemia negatively interfered with HDL-C. The HDL-C and immunoglobulin G (IgG) levels were measured before and after liver transplantation. The patient had high IgG with polyclonal hypergammaglobulinemia and an undetectable HDL-C before transplantation. Falsey undetectable HDL-C levels were ruled out by two easy methods: sample dilution and back calculation based on Friedewald formula. This false negative value of HDL-C was also confirmed with lipoprotein electrophoresis. One must be careful in hyperimmunglobulinemic patients when using direct homogeneous HDL-C assay.

Key Words: Hepatitis, autoimmune; cholesterol, HDL; hypergammaglobulinemia


Anahtar Kelimeler: Otoimmün hepatit;olesterol, HDL; hypergammaglobulinemi


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utoimmune hepatitis is a chronic necroinflammatory liver disease characterized by massive infiltration of lymphocytes and plasmocytes in the portal tract and serologically high titers of anti-smooth muscle antibody (ASMA), anti-ds DNA antibody, cytoplasmic-antineutrophil cytoplasmic antibodies (C-ANCA), antinuclear antibody (ANA) or antimitochondrial antibody (AMA), and high globulin levels.1 The liver plays a major role in the regulation of fatty acid, triglyceride, and cholesterol metabolisms. Severe liver injury as in cirrhosis, often leads to a decrea-
Dyslipidemia is routinely evaluated by measurement of a profile including total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and triglyceride concentration measurements. Many analytical techniques including ultracentrifugation, electrophoresis, precipitation based methods have been used for measuring HDL-C and currently direct homogeneous assays are more preferable. Although all of these methods show close agreement with recommended reference method (ultracentrifugation with HDL-C measurement after precipitation of apoprotein B-containing lipoproteins), they may show some differences. As an advantage of direct method, manual precipitation steps are not necessary and there is less interference with triglycerides.

The objective of this study is to show the interference of polyclonal hypergammaglobulinemia with false-negative HDL-C using direct homogeneous method in an 18-year-old female with cirrhotic process of autoimmune hepatitis.

**CASE REPORT**

An 18-year-old female with 158 cm height and 60 kg weight admitted to transplantation division of Ege University Medical School Hospital in April 2007 because of abdominal distension, jaundice and drowsiness.

She presented with fatigue, sickness, vomiting and jaundice in 1997. The laboratory test results at that time were shown in Table 1. Advanced diagnostic investigations had been performed. Anti-smooth muscle antibody and anti-ds DNA antibody were positive (1/5120, 1/121 respectively), and cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA), antinuclear antibody (ANA), antimitochondrial antibody (AMA) were negative. Anti HAV, HBs Ag, HBe Ag, Anti Hbe, Anti Hbc IgM and IgG, Anti Hbc, Anti HBs Ag, Anti HCV IgG, Anti Delta IgG, EBV VCA IgG, Anti HIV1 and HIV2 were all negative. An atrophic liver, enlarged spleen and ascites were observed in the upper abdominal ultrasonography. Hepatic biopsy was compatible with severe necrotic inflammatory hepatic degeneration with cirrhotic process, and HBs Ag and HCV immunohistological staining were negative. In her family history, there was no history of the hepatic disease, but her mother had autoimmune thyroiditis. In the light of all these data, her diagnosis was autoimmune hepatitis.

She received 30 mg/day corticosteroid treatment between 1997-1998 and 5 mg/day corticosteroid treatment between 1999-2005. Cyclosporine therapy of 50 mg/day was administered for four years. In 2005, she had nasal and gingival bleeding because of trombocytopenia. She had 2 second grade oesophageal varicose veins and endoscopic variceal ligation was performed in 2006.

She admitted to our university hospital for hepatic transplantation in April 2007, at the decompensation period of end stage cirrhosis. Her laboratory test results on admission are given in Table 1.

On physical examination, her vital signs were normal. Blood pressure was 130/70 mmHg and heart rate was 100/min. Her body temperature was 36.6°C. The conjunctiva as jaundiced, heart sounds were normal and breath sounds were diminished at the base of the lungs on chest auscultation. The liver was not palpable and there was splenomegaly. Edema was significant on lower legs and feet. She recieved a living donor transplant liver from her aunt in April 2007.

**MEASUREMENT METHODS**

TC, triglyceride, HDL-C, LDL-C, alanine aminotransferase, aspartate aminotransferase, total bilirubin, direct bilirubin, γ-glutamyltransferase, alkaline phosphatase, total protein, albumin, apolipoprotein A-1 and apolipoprotein B analyses were performed on Modular system (MODULAR® ANALYTICS Roche, Diagnostics GmbH, Mannheim, Germany) before and 7 days after the transplantation. Immunoglobulin concentrations were measured by immunoturbidimetric assays (Tina-quant IgG Gen.2, Tina-quant IgM Gen.2, Tina-quant IgA
The HDL-C and the LDL-C were both 2nd generation homogeneous direct enzymatic colorimetric quantitative assays (Roche Diagnostics GmbH, Mannheim, Germany).

Serum lipoprotein electrophoresis (SAS-MX lipoprotein Helena Biosciences Europe, cat no:101200), protein electrophoresis (SAS-MX Serum protein Helena Biosciences Europe, cat no:100100) and immunofixation electrophoresis (SAS-MX Immunofixation Helena Biosciences Europe, cat no:100300) were performed by agarose gel based kits on admission and 7 days after the transplantation.

Informed consent was obtained from the patient.

ADDITIONAL INVESTIGATIONS

Lipoprotein electrophoresis is shown in Figure 1. Low but clearly detectable prebeta (very low density lipoprotein), beta (LDL) and alpha (HDL) bands were also concordant with low serum TC and triglyceride concentrations.

The presence of hypergammaglobulinemia was documented by measuring total protein, albu-
min and calculating the globulins (total protein – albumin = globulins; in this case, 97 -19 = 78 g/L).

The patient was in the cirrhotic process of autoimmune hepatitis in which polyclonal hypergamma-globulinemia is likely to be encountered. As a consequence of elevated total protein combined with low albumin level and hypergamma-globulinemia; sequential serum protein electrophoresis, quantitative immunoglobulin analysis and immunofixation electrophoresis were performed before and 7 days after transplantation. Before the transplantation, protein electrophoretic profile was showed a pattern with a broad polyclonal band in the gamma region (Figure 2a). Polyclonal hypergamma-globulinemia refers to an increase in structurally different one or more immunoglobulins (polyclonal) or fragments of one immunoglobulin protein.4 In the immunofixation electrophoresis, IgG was the most abundant polyclonal protein and no monoclonal gammopathy was detected (Figure 3). After the transplantation, the broad polyclonal gamma band vanished in the electrophoretogram (Figure 2b).

Normally, in the HDL-C measurement, the absorbance is low at the beginning of the reaction. After adding the color reagent the absorbance increases, and HDL-C concentration is calculated by calculating the difference between the initial and the final absorbances. In this case, a very high absorption was present at the beginning compared to a protein electrophoresis of a normal person (Figure 4a, 4b). This high absorptivity was probably a result of precipitation of gammaglobulins which caused turbidity in serum sample. At the end of the reaction, the absorption decreased however, in a normal person it is expected to increase. As a result, this patient with polyclonal hypergamma-globulinemia had a false-negative HDL-C value. The HDL-C result remained negative when the test was repeated and serial dilutions were performed (x2, x4). A positive but very low result was obtained after diluting the serum five fold (Figure 4c). On the 7th day of transplantation, a positive HDL-C concentration was measured (Figure 4d). All the reaction kinetics of HDL-C is shown in Figure 4. The serum sample had no visual turbidity or an abnormal appearance of serum lipemia or hemolysis, and the icterus index was 2.

**DISCUSSION**

Negative interference in HDL-C measurement appears in patients with monoclonal gammopathies.5-8 In one study by Zapico et al four HIV infected patients with polyclonal hypergamma-globulinemia had falsely low HDL-C results.9 Likewise, our autoimmune hepatitis case had a polyclonal hypergamma-globulinemia with undetectable HDL-C levels.

On the serum lipoprotein electrophoresis, alpha-lipoprotein (HDL-C) fraction was detectable, and apolipoprotein A-1 was measured as 0.34 g/L. Warnick et al showed that electrophoretic systems could obtain acceptable quantification of HDL-C.3 Another supporting data is that, after diluting out the serum, the reaction kinetics of HDL-C has changed. In the reaction kinetics graph of another patient with normal protein electrophoresis, the initial absorbance (after the addition of reagent 1) at 600 nm was low, and increased after adding the reagent 2 (Figure 4a). In our case, the initial absorbance was much higher after adding reagent 1. Since the initial absorbance was very high, the value calculated for HDL-C by subtracting the initial from the final absorbance was ended with a negative HDL-C value (Figure 4b). Diluting out the serum for two or for four times was not eno-
ugh, and HDL-C concentration remained undetectable, but after diluting out the serum for five times, the initial absorbance decreased approximately 90% and a positive but very low concentration could be measured (Figure 4c).

All these data strongly suggested an interference due to a polyclonal hypergammaglobulinemia in the direct HDL-C method. In Zapico-Muñiz et al study, falsely low HDL-C concentrations were detected in four HIV infected patients with polyclonal hypergammaglobulinemia, and they presumed that interaction between dextran sulfate (the first reagent used in the direct HDL-C method) and immunoglobulins lead to large complex formation or an immunoglobulin induced aggregation cause high light dispersion.

As Pang et al suggested, to tackle with the analytic problems with the direct HDL-C assay by an alternative approach in case of atypical sera with monoclonal and polyclonal hypergammaglobulins, Friedewald formula was incorporated into the directly measured LDL-C and triglyceride values. Using this back calculation for HDL-C based on Friedewald formula, our patient’s HDL-C concen-
Concentration was approximately 0.52 mmol/L. This is an alternative and a simple way to estimate the HDL-C concentration in sera with suspected immunoglobulin interference.

On 7th day after the transplantation, the patients' HDL-C was found to be 0.13 mmol/L by direct measurement and 0.23 mmol/L by back calculation. Serial dilutions (x2, x5) were performed to evaluate the interference of hypergammaglobulins. The results remained undetectable suggesting that there was no interference. In the reaction kinetics of HDL-C the first absorbance was very low and it increased later to give a positive result (Figure 4d). The decreased concentrations of IgG, IgA and IgM also suggested that the suspected immunoglobulin interference disappeared.

In conclusion, undetectable HDL-C result was observed by direct homogeneous method in a polyclonal hypergammaglobulinemic patient with autoimmune hepatitis in cirrhotic process. False undetectable HDL-C levels were ruled out by two easy methods: sample dilution and back calculation based on Friedewald formula. In patients with polyclonal hypergammaglobulinemia, HDL-C results by direct homogeneous method should be interpreted with care, and practical methods not interfering with other biochemical abnormalities should be used.

FIGURE 3: Immunofixation electrophoresis before the liver transplantation. G: anti-IgG antibodies; A: anti-IgA antibodies, M: anti-IgM antibodies, K: anti-kappa chain antibodies, λ: anti-lambda chain antibodies, respectively. IgG, kappa and lambda light chains are polyclonal.

FIGURE 4: Reaction kinetics of HDL-C of a patient with normal protein electrophoresis (4a), our case before liver transplantation (4b), after dilution for five times (4c), 7 days after hepatic transplantation (4d) HDL-C is measured by enzymatic reaction steps; hydrolyzing cholesterol esters, oxidizing the free cholesterol's 3-OH group to ketone, yielding H2O2, and measuring peroxidase catalyzed reaction in which a dye is formed. The color intensity is directly proportional with the HDL-C concentration. Vertical arrows indicate the time points at which the reaction follows as; S: sample addition, R1: first reagent addition, R2: second reagent addition, 1st: First absorbance measurement, 2nd: Second absorbance measurement.
REFERENCES


