A case report: When autoantibodıes are together with iron overload, do not judge a book by its cover

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Abstract

The presence of autoantibody positivity together with elevated ferritin level and high transferrin saturation can cause diagnostic dilemma. We encountered a challenging case of 38-year-old male patient with new-onset diabetes, malaise, weight lose, dark-yellow discoloration of skin and splenomegaly. Initial laboratory tests revealed thrombocytopenia, leucopenia, elevated unconjugated bilirubin level and mildly elevated liver enzymes in cholestatic pattern. Anti-nuclear antibody(ANA) and anti-smooth muscle antibody(ASMA) were positive with titers of 1/160 and 1/320 respectively along with hypergammaglobulinemia. Transferrin saturation was 92%, and ferritin was 498 g/L. HFE gene mutation analysis showed C282Y heterozygote mutation which is not diagnostic, but supporting HH. As liver biopsy is the most accurate way to differentiate autoimmune hepatitis(AIH) and hereditary

hemochromatosis(HH), it was applied. The diagnosis of HH was confirmed, and of AIH was ruled out. This case highlighted the importance of paying attention to all findings to avoid misdiagnosis and treatment which might result in dangerous outcomes. Additionally, inspite of genetic test, the liver biopsy has still crucial importance by guiding to reach the accurate diagnosis in patients with iron overload especially in patients with concomitant autoantibody positivity.

Keywords: Iron overload, hemochromatosis, autoimmune hepatitis, elevated transferrin saturation

Abbreviations

AIH	Autoimmune Hepatitis
ANA	Anti-Nuclear Antibody
ASMA	Anti-Smooth Muscle Antibody
HAI	Hepatic Activity Index
HFE	Human Homeostatic Iron Regulator Protein
HH	Hereditary Hemochromatosis
MRI	Magnetic Resonance Imaging
TS	Transferrin Saturation

Introduction

Iron overload can cause diagnostic dilemma in patients with chronic liver disease. Abnormal serum iron studies implying iron overload can be either primary as in the hereditary hemochromatosis(HH) or secondary to chronic inflammation associated with chronic liver diseases such as alcoholic liver disease, non-alcoholic

fatty liver disease, chronic viral hepatitis and, even –very rarely– autoimmune hepatitis(AIH)(1). HH is characterized by abnormal accumulation of iron not only in parenchymal cells of liver, but also in endocrine glands, heart and skin(2). Whereas, secondary iron accumulation mostly affects reticuloendothelial cells. To differentiate primary and secondary iron overload, extensive diagnostic workup may be required.

The circulating autoantibodies may be useful for the diagnosis of various autoimmune disorders including AIH. These autoantibodies may also be incidentally discovered in healthy individuals(3). The increased prevalance of concurrent autoantibody positivity in patients with other chronic liver diseases including non-alcoholic liver disease, chronic hepatitis C and drug-induced liver injury is also a well-known clinical entity. However, the possible linkage between hemochromatosis and predisposition to autoimmunity is still under investigation(4, 5). Previously, a limited number of patients cases of AIH together with abnormally high transferrin saturations and ferritin levels were reported(6-10). On the other hand, physicians should also be aware of that coincidental autoantibody positivity and hypergammaglobulinemia may mask the underlying insidious HH, and may lead to misdiagnosis of AIH which is more prevalent than HH.

Herein, we report a cirrhotic patient with iron overload who has also new-onset diabetes, hypergammaglobulinemia as well as high titers of autoantibodies.

Case Presentation

A 38-year-old male patient, with new-onset diabetes, has been referred to Gastroenterology clinic because of bicytopenia. His chief complaints were malaise and weight lose. At physical examination, there were dark-yellow discoloration of skin and splenomegaly. He denied any alcohol, illicit drug or herbal product use. He had no

significant family history for liver diseases. Laboratory tests revealed thrombocytopenia, leucopenia, elevated unconjugated bilirubin level, and mildly elevated liver enzymes in cholestatic pattern(ALT/ALP ratio <2) (Table 1). Viral hepatitis(hepatitis A, B, C and E) was ruled out with negative viral panel. Anti-nuclear antibody(ANA) and anti-smooth muscle antibody(ASMA) were positive with titers of 1/160 and 1/320 respectively along with hypergammaglobulinemia. Transferrin saturation was 92% and ferritin was 498 g/L. MRI revealed cirrhotic liver with multiple siderophilic nodules, portal vein thrombosis, atrophic pancreas, splenomegaly, splenorenal shunt and esophageal varices. The liver biopsy was done inspite of trombocytopenia, and revealed mild lymphocytic inflammatory infiltrate in portal areas, local piecemeal necrosis and hepatocyte-predominant iron deposition (Figure1). Modified HAI score was 3/18 and fibrosis score was 5/6. C282Y heterozygote mutation was detected at HFE gene mutation analysis.

Discussion

The case reported here was a diagnostic challenge putting the physician into a dilemma between HH and AIH due to the presence of both iron overload and strong autoantibody positivity. The liver biopy was to key resolving the conflict by confirming HH and ruling out AIH.

HH is the most commonly identified genetic disorder in Caucasions, with the highest prevalence among the populations of northern European descent. The underlying pathophysiological mechanisms include decreased expression of hepcidin and increased absorption of dietary iron as a result of gene mutations. The identified main genetic defect is C282Y homozygote mutation in *HFE* gene, accounting for 80-

85% of HH patients (*HFE*-related HH). However, because of variable penetrance, less than 10% of people carrying C282Y homozygote mutation encounter end-organ damage. Other two important HFE gene mutations are H63D and S65C mutations which are mostly detected as compound heterozygocity, meaning that C282Y mutation is present in one allele and H63D or S65C mutation is present in other(1). Clinical presentations develop much less frequently in patients having compound heterozygote mutations compared to those with C282Y homozygote mutation(11). Additionally, mutations in other genes coding several proteins of iron metabolism leading to the hereditary iron overload syndromes(non-*HFE* related HH) have been identified over the last 20 years(1, 11). Although the high frequency of C282Y mutation in Europe allows to base the diagnostic algorithm on mutation analysis, *HFE*-related HH is very rare in Turkey. C282Y mutation could not be detected neither in the general population nor among HH patients in Turkey (Table 2)(12, 13). Therefore, we belive that HFE gene mutation analysis may not be a reasonable approach as the first step of HH diagnostic evaluation in some populations. HFE gene mutation analysis was applied in our case due to high clinical suspicion for HH. The presence of C282Y heterozygote mutation was a supportive finding by considering the rarity of HFE mutation in Turkish population.

Secondary iron overload is much more common clinical condition than HH. The differential diagnosis has a wide range of diseases including but not limited to iron-loading anemias and parenteral iron treatment and chronic inflammation associated with other chronic liver diseases like chronic viral hepatitis, alcoholic liver disease and non-alcoholic liver disease(1). Elevated ferritin levels as a positive acute-phase reactant together with normal transferrin saturation can be seen in AIH, but AIH

associated with iron overload is an extremely rare condition. To our knowledge, there are only 5 cases of AIH previously reported with an elevated ferritin level and high transferrin saturation. In 3 of them, HH was strongly considered due to abnormally high transferrin saturations and ferritin levels (case 1 with TS 89%, ferritin 1570 g/L, negative HFE gene mutation (6); case 2 with TS 91%, ferritin 2463 g/L, HFE gene mutation analysis not applied(7); case 3 with TS 78%, ferritin 1800 g/L, H63D heterozygote mutation(8)), but liver biopsies revealed only AIH in all of them. Case 4 was a patient with liver failure and hyperferritinemia(ferritin 16900 g/L), and AIH was diagnosed by liver biopsy(9). Case 5 had diagnosed of simultaneous AIH and HH, since patient had TS %94, ferritin 8190 g/L, positive HFE gene mutation along with positive ASMA at a titer of 1/640, and the liver biopsy showed severe sideronecrosis and plasmacytosis with acute hepatocyte damage(10).

The coexistence of HH and autoimmune conditions is an area of investigation, and it is suggested that HFE gene mutations may predispose patients with HH to autoimmune diseases(5). This predisposition may lead to coincidental positivity of liver spesific autoantibodies, and so misdiagnosis of AIH. In our case, autoantibody positivities at high titers and hypergammaglobulinemia created a diagnostic dilemma, even though HH was strongly suspected because of concomitant presence of new-onset diabetes and skin pigmentation in a young male patient with cirrhosis. Furthermore, the pancreatic atrophy resulting in diabetes was attributed to iron accumulation rather than immune causes. HH was diagnosed based on high ferritin level associated with markedly increased transferrin saturation, and also demonstration of iron accumulation in liver by MRI. The liver biopsy not only confirmed primary iron accumulation in the hepatocytes, but also excluded AIH.

As a conclusion, this case was a diagnostic challenge emphasizing the importance of paying attention to all clinical and laboratory findings in order to avoid misdiagnosis of AIH in patient with iron overload and autoantibody positivity. Since the management of AIH and HH are completely different, the misdiagnosis of AIH or missing the diagnosis of HH might result in untoward outcomes in this patient. We belive that the liver biopsy plays crucial roles to reach the accurate diagnosis in the presence of both autoantibody positivity and elevated ferritin level together with high transferrin saturation.

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Tables:

Table 1. Laboratory Findings at Admission

Tests	Values	Laboratory Reference Ranges
Complete Blood Count		
WBC	**2.6 x 10^3/µL**	4.3-10.3 x 10^3/µL
RBC	**3.92 x 10^3/µL**	4.38-5.77 x 10^3/µL
Hemoglobin	14.8 gr/dL	13.6-17.2 gr/dL
Platelets	**59 x 10^3/µL**	156-373 x 10^3/µL
Biochemical Tests		
Sodium	135 mEq/L	136-146 mEq/L
Potassium	3.87 mEq/L	3.5-5.1 mEq/L
Blood Urea Nitrogen(BUN)	8.2 mEq/L	6-20 mEq/L
Creatinine	0.38 mEq/L	0.67-1.17 mEq/L
Total Bilirubin	**4.22 mg/dL**	**0.3-1.2 mg/dL**
Direct Bilirubin	**1.04 mg/dL**	**0-0.2 mg/dL**
Indirect Bilirubin	**3.18 mg/dL**	**0-1.2 mg/dL**
ALT	49 U/L	<50 U/L
AST	**75 U/L**	<50 U/L

ALP	**153 U/L**	30-120 U/L
GGT	**59 U/L**	<55 U/L
Lactate Dehydrogenase	**344 U/L**	<248 U/L
Total Protein	7.63 gr/dL	6.4-8.3 gr/dL
Albumin	**3.13 gr/dL**	3.5-5.2 gr/dL
Serology/Immunology		
IgG	**3180 mg/dL**	751-1560 mg/dL
IgA	307 mg/dL	82-453 mg/dL
IgM	140 mg/dL	46-304 mg/dL
ANA	**Positive, 1/160**	Negative
ASMA	**Positive, 1/320**	Negative
Liver/kidney microsomal antibody(LKM)	Negative	Negative
Anti-mitochondrial antibody(AMA)	Negative	Negative
Other Biochemical Tests		
Iron	**234 µg/dL**	50-150 µg/dL
Total Iron Binding Capacity	254 µg/dL	228-428 µg/dL
Transferrin Saturation	**92%**	20-50%
Ferritin	**498.7 µg/L**	20-336 µg/L
HbA1c	8.3%	
Prothrombin Time(INR)	**1.29**	0.8-1.2
aPTT	**35.5 seconds**	22.5-32 seconds
Ceruloplasmin	28.6 mg/dL	22-58 mg/dL

Table 2. *HFE* gene mutation analysis studies in Turkey

	Study Population	C282Y/ C282Y	C282Y / N	H63D/ H63D	H63D / N	C282Y/ H63D
Barut G 2003(14)	26 healthy volunteers with TS ≥ 50% in fasting state	0	0	1 (M)	10(M), 1(F)	0
Bozkaya H 2004(15)	65 blood donors with UIBC <28 microM	0	0	0	7	0
Simsek H 2004(13)	86 healthy blood donors with TS≥ 45% 57 healthy blood donors with TS< 45%	0 0	0 0	11 0	25 0	0 0
Simsek H 2005(12)	5 patients with HH	0	0	0	5	0
Simsek H 2006(16)	30 patients with nonalcoholic steatohepatitis			1	12	
Yonal O 2007(17)	Family screening after a diagnosis of C282Y homozygote HH	2	1	0	3	3
Dulger AC 2012(18)	159 healthy men			3	11	

Karaca H 2013(19)	2304 participants	1	1	0	0	1
Unal S 2014(20)	87 beta-thalassemia major, 13 beta-thalassemia intermedia patients and 100 healthy blood donors	0	0			
Ozturk S 2007(21)	141 healthy adults	0	0	2	30	0

TS: Transferrin saturation, UIBC: Unbound iron binding capacity, HH: Hereditary hemochromatosis, M: Male, F: Female

Figure Legends:

Fig 1. (A) Brown pigments can be seen in liver biopsy with hemotoxylin&eosin stain(x200). (**B)** Excessive iron deposition is demonstrated by Prussian blue staining(x400).

Figures: