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Diagnosis and treatment difficulties in 18-year-old male patient with hereditary hemochromatosis, chronic hepatitis B, Gilbert syndrome and ulcerative colitis

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Among possible causes of chronic hepatitis in adolescents most common are infections, autoimmune disorders and metabolic diseases. Thus, diagnostic procedures should be multidirectional. This study reports diagnosis and treatment difficulties in an 18-year-old male patient with hereditary hemochromatosis (HH), ulcerative colitis (UC), chronic hepatitis B (CHB) and Gilbert syndrome. The presented case illustrates problems in diagnostics related to the presence of numerous disease conditions in one patient. It should be taken into consideration that these diseases coexisting in one patient can mutually affect their symptoms creating specific diagnostic difficulties.

Keywords: hereditary hemochromatosis, ulcerative colitis, chronic hepatitis B, Glibert syndrome

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INTRODUCTION

Among possible causes of chronic hepatitis in adolescents most common are infections, autoimmune disorders and metabolic diseases. Thus, diagnostic procedures should be multidirectional.

This study reports diagnosis and treatment difficulties in an 18-year-old male patient with hereditary hemochromatosis (HH), ulcerative colitis (UC), chronic hepatitis B (CHB) and Gilbert syndrome.

CASE REPORT

An 18-year-old male was admitted to the Department of Infectious Diseases with the diagnosis of chronic hepatitis B. His past medical history included ulcerative colitis that was diagnosed and treated at the age of 16 years in the Department of Pediatric Gastroenterology, Hepatology and Nutrition. He was admitted to this hospital due to repeated episodes of diarrhea with rectal bleeding. The diagnosis was established after colonoscopy and histopathological examination of specimens from the colon. In spite of recurrent rectal bleeding, the patient did not present anemia or iron deficiency (laboratory parameters are presented in Table 1).

Additionally, the patient was HBsAg positive, HBeAg negative, hepatitis B virus (HBV) infection had been observed since he was 7 years old. Most probably he was infected during infancy. At the age of 7 years liver biopsy was performed and minimal hepatitis was diagnosed. Serum ALT and AST activities were in the normal range. No treatment was administered. There was a family history of asymptomatic HBV infection in his father and mild unconjugated hyperbilirubinemia in his younger brother.

At the beginning his ulcerative colitis treatment consisted of mesalamine (5-aminosalicylic acid, 5-ASA) and corticosteroids rectally, but no remission was achieved. At the age of 17 years, azathioprine was added and initially good tolerance of this therapy with normalization of stools was observed.

After 5 months of this treatment the patient was admitted to the hospital due to weakness, jaundice, abdominal pain, vomiting and weight loss of 7 kg. On physical examination, he presented hepatomegaly with tenderness in the right epigastrium. Laboratory investigations revealed neutropenia, rise of aminotransferase activities (ALT 421 IU/l, AST 388 IU/l) and elevated serum total bilirubin with its conjugated fractions (total bilirubin 8.56 mg/dl, conjugated bilirubin 2.95 mg/dl). Initially azathioprine-induced toxicity or exacerbation of chronic hepatitis B were suspected. The presence of HBV DNA in blood was confirmed but it did not exceed 100 000 IU/ml. Serum activity of thiopurine methyltransferase was within normal range — 9.36 nmol/ml per h. No abnormalities were found in abdominal US imaging. Additional laboratory tests excluded hepatitis C infection, autoimmune hepatitis and Wilson's disease (laboratory parameters are presented in Table 1).

In the liver biopsy features of portal, periportal and intralobular chronic hepatitis were found. Moderate iron deposits were present in hepatocytes. Biochemical serum iron indices were also moderately elevated. Diagnosis of hereditary hemochromatosis was established after confirmation of the C282Y/C282Y muta-

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Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; 5-ASA, 5-aminosalicylic acid; CHB, chronic hepatitis B; EASL, European Association For the Study of the Liver; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HH, hereditary hemochromatosis; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; PSC, primary sclerosing cholangitis; UC, ulcerative colitis

Table 1. Laboratory parameters at admission of a patient to the Department of Infectious Diseases

Laboratory parameters	Result	Normal range
Hemoglobin (g/l)	13.9	14.0–18.0
Hematocrit (%)	39.8	38.0–54.0
RBC (T/l)	3.7	4.0–6.0
WBC (G/)l	2.76	4.00–10.00
Neutrocytes (G/l)	3.86	1.90–8.00
PLT (G/l)	297	130–440
ALT (U/l)	421	5–41
AST (U/l)	388	5–37
GGTP (U/l)	92	8–61
ALP (U/l)	121	<390
Total bilirubin (mg/dl)	8.56	<1.2
Conjugated bilirubin (mg/dl)	2.95	<0.30
IgG (g/l)	12.09	5.36–15.47
ANA	Negative	1:<10
SMA	Negative	1:<10
LKM	Negative	1:<10
pANCA	Negative	1:<10
Ceruloplasmin (g/l)	0.457	0.15–0.30
Copper in 24-hour urine (μg/24h)	37	<60
Total protein (g/l)	7.21	64–83
Albumin (g/l)	5.83	3.0–5.0
Alfa1globulin (%)	6.5	2.9–4.9
Alfa2globulin (%)	6.0	7.1–11.8
Betaglobulin (%)	12.6	7.9–13.7
Gammaglobulin (%)	16.6	11.1–18.8
Iron (μg/dl)	211	60–170
Transferrin sat. (%)	66	15–50
Ferritin (ng/ml)	492	18–250
HBsAg	Positive	Negative
HBeAg	Negative	Negative
Anti-HBe	Positive	Negative
HBV DNA in blood (IU/ml)	75000	Negative
Anti-HCV	Negative	Negative
HCV RNA	Negative	Negative

tion of the *HFE* gene. This genotyping was performed by PCR and restriction fragment length polymorphism (PCR-RFLP) analysis (Fig. 1) as described previously (Sikorska *et al.*, 2008). Due to permanent unconjugated hyperbilirubinemia, Gilbert syndrome was suspected. *UGT1A1* gene polymorphism was assayed by melting curve analysis of PCR-amplified genome fragments containing the site of mutation with fluorescent molecular probes (Fig. 2) as described elsewhere (Romanowski *et al.*, 2009). Both homozygotic mutations UGT1A1*60 and UGT1A1*28, which are responsible for impairment of bilirubin glucuronisation and often co-occur, were detected.

DISCUSSION

The symptoms of chronic hepatitis warrant diagnostics directed to infectious, autoimmune and metabolic diseases. Hepatobiliary diseases are rather common in patients with inflammatory bowel diseases (IBD). The most frequent are primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) (Knight & Murray, 2009). In the presented case, these diagnoses were excluded based on immunological analysis and liver biopsy. Diagnosis of HH type 1 was established eleven years after CHB and two years after UC diagnosis.

HH type 1 associated with *HFE* gene mutations is not considered a disease of the childhood or adolescence. Biochemical abnormalities of iron metabolism precede symptoms of tissue iron overloading that may finally lead to irreversible multiorgan damage. Although *HFE* gene mutations are detected with relatively high frequency in Caucasians (1:200–1:400), clinical penetration of HH type 1 is much lower (Bomford, 2002). Recently, results of a prospective 12-year study demonstrated that only 28.4% of male and 1.2% of female C282Y/C282Y homozygotes presented clinical symptoms of iron-overload-related disease (Allen *et al.*, 2008). The reasons for the low penetration of HH type 1 genotype include dietary habits, alcohol intake, toxicity of drugs, coexistence of chronic hepatitis C, but it seems that these factors do not explain the observed differences in expression of the disease in all C282Y/C82Y homozygotes (Beutler, 2003). Hepcidin, a central peptide regulator of iron homeostasis, is considered to play an important role in the development of hereditary hemochromatosis phenotype. Iron stores and inflammation are known inducers of hepcidin that acts as a negative regulator of iron absorption in the small intestine and iron release from macrophages (Nicolas *et al.*, 2001; Pigeon *et al.*, 2001; Nemeth *et al.*, 2003). Impairment of hepcidin expression and disruption of hepcidin regulation has been demonstrated in HFE-related hemochromatosis (Bridle *et al.*, 2003; Kulaksiz *et al.*, 2004). Measurement of hepcidin expression was not possible in our case and we cannot show that the chronic inflammatory state related to UC influenced hepcidin expression and decreased iron absorption. Moreover, the role of hepcidin, a beta-defensin-like peptide, in the analysed case seems to be much more complex. Defensin deficiency is implicated in the pathogenesis of inflammatory bowel diseases. It might be suggested that decreased expression of hepcidin related to *HFE* gene mutation could influence development of UC symptoms (Verga Falzacappa & Muckenthaler, 2005; Arnold *et al.*, 2009; Ramasundara *et al.*, 2009).

In the presented case, symptoms of HH were unexpected. Both biochemical and tissue marks of iron overload were diagnosed at the age of 18 during long-lasting exacerbation of ulcerative colitis, e.g., chronic inflammatory disease with recurrent rectal bleeding. Anemia is a very frequent systemic complication of inflammatory bowel disease and its diagnosis and treatment should not be neglected in patients with these diseases (Gisbert & Gomollon, 2008). In the presented patient blood cell morphology and serum iron indices were carefully checked to exclude potential iron deficiency rather than its excess. Moreover, in the case of recurrent blood loss, no signs of iron overload should be expected. It appears that in this case the dysfunction of HFE protein due to the C282Y/C282Y *HFE* gene mutation resulting in accumulation of iron protected against anemia. This factor caused advantageous modulation of the clinical course

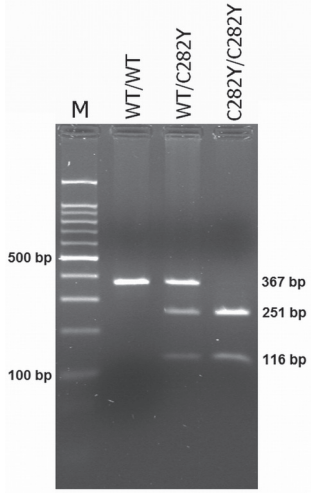

Figure 1. Determination of C282Y mutation of *HFE* gene by PCR-RFLP analysis
M, 100 bp DNA ladder

of ulcerative colitis. The HLA region is implicated in pathogenesis of ulcerative colitis. Specific HLA-DR alleles are known to influence the phenotype of the disease (Hampe *et al*., 1999; Stokkers *et al*., 1999; Walters & Silverberg; 2006). It could be speculated that HLA-DR alleles located on the short arm of chromosome 6 near the locus of the *HFE* gene may influence penetration of hereditary hemochromatosis type 1. A stronger phenotypic expression of *HFE* gene mutations caused by the presence of specific HLA-DR genes would protect against anemia and become an advantageous modulator of UC clinical course (Ponsioen *et al*., 2001; Beutler *et al*., 2003). Additionally, in this patient and his younger brother mild unconjugated hyperbilirubinemia was diagnosed. Based on molecular investigations the diagnosis of Gilbert syndrome was established. The recessive polymorphism A(TA)$_7$TAA (UGT1A1*28) in which seven instead of six TA repeats are found in the TATA box of bilirubin uridine diphosphate glucuronyl transferase gene (*UGT1A1*) is responsible for most cases of Gilbert syndrome (Bosma *et al*., 1995). It often co-occurs with the homozygosity of the T to G substitution in the *UGT1A1* gene start codon (c.-3279T>G, also named UGT1A1*60) (Maruo *et al*., 2004). Although responsible for benign hyperbilirubinemia, this polymorphism may impair biotransformation of some drugs thus increasing the toxicity of xenobiotics requiring glucuronidation (Lankisch *et al*., 2006; Biason *et al*., 2008).

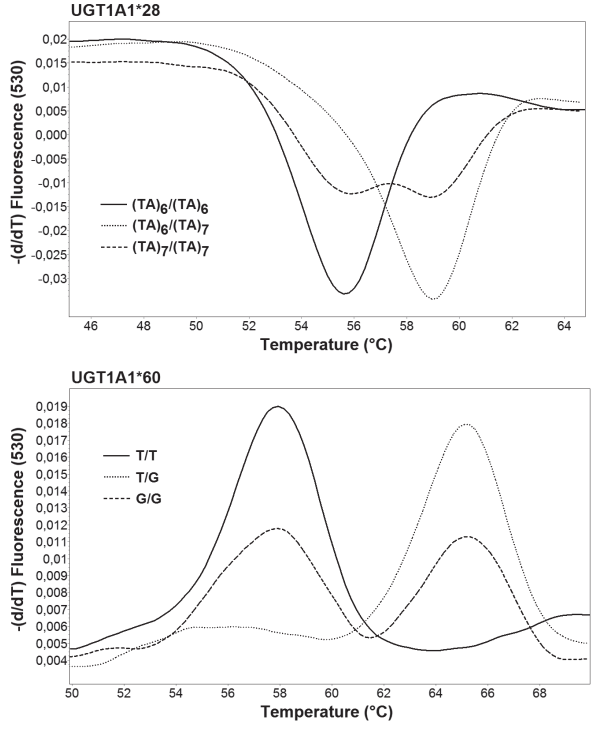

Figure 2. Genotyping of UGT1A1*28 (seven TA repeats in TATA box of *UGT1A1* gene) and UGT1A1*60 (c.-3279T>G) polymorphisms performed by fluorescent molecular probes assays

The effect of *UGT1A1* gene polymorphism and mild hyperbilirubinemia on iron-overload-related disease is unknown. Location of the *UGT1A1* gene on chromosome 2 excludes the concept of nearby genes affecting HFE-related hemochromatosis expression. There are several reports indicating strong antioxidant properties of bilirubin (McCarthy, 2007; Ollinger *et al*., 2007). Thus, elevated serum bilirubin might diminish oxidative stress and prevent organ damage caused by iron accumulation. Recently, Romanowski *et al*. (2009) proposed a different mechanism of bilirubin action on iron overload. Based on the observations of patients with HH and mutations of *UGT1A1* gene they concluded that elevated levels of bilirubin might promote iron loading by decreasing oxidative stress and inhibiting hepcidin expression signalling due to lower inflammatory activity (Nicholas *et al*., 2002).

Unlike in chronic hepatitis C, the presence of liver iron deposits and abnormalities of iron metabolism are not extensively studied in chronic hepatitis B. Mild or moderate iron overload not associated with *HFE* gene mutations probably is not very rare in patients infected with HBV, but sufficient data from different populations is lacking. As in other chronic liver diseases, iron causes tissue injury through oxidative stress (Martinelli *et al*., 2004). Liver iron deposits that may accompany high necroinflammatory activity not only promote fibrogenesis resulting in accelerated progression to liver cirrhosis but also increase the risk of hepatocellular carcinogenesis. A synergistic, unfavourable effect of hereditary hemochromatosis and chronic viral hepatitis is therefore observed and urges application of appropriate, effective treatment. The course of CHB in the presented case was mild as it is often observed in HBV infection acquired in early childhood. Increased HBV replication and following intensification of necroinflammatory process due to immunosuppression or drug hepatotoxicity have been proposed to explain liver disease exacerbation observed during therapy of UC with azathioprine. This mechanism of liver damage was questionable in the presented patient because his serum activity of thiopurine methyltransferase was within the normal range — 9.36 nmol/ml/h (normal range >5 nmol/ml per h) (Gisbert *et al*., 2001).

Although exacerbation of liver disease observed in the presented case during azathioprine treatment could result in iron deposition in the liver, it should rather be found in macrophages as a result of necroinflammatory hepatocytic damage (Deugnier *et al*., 2008). However, iron deposits were detected in hepatocytes indicating primary character of iron loading as it is observed in HH. The patient is at present subjected to antiviral treatment with nucleoside analogues according to EASL recommendations (EASL, 2009). Interferon will not be administered because of high risk of exacerbation of ulcerative colitis.

Phlebotomy is considered in the treatment of HH but now it is to be adjourned until antiviral therapy is effective and evaluation of iron metabolism parameters after inhibition of viral replication becomes possible.

The presented case illustrates problems in diagnostics related to the presence of numerous disease conditions in one patient. It should be taken into consideration that these diseases coexisting in one patient can mutually affect their symptoms creating specific diagnostic difficulties.

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