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Homozigotni oblik nasljedne hemokromatoze u bolesnika s beta-talasemijom minor: prikaz slučaja

Homozygous form of hereditary hemochromatosis in a patient with beta-thalassemia minor: case report

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Sažetak

Uvod: Dijagnostički pristup bolesniku koji istodobno boluje od nasljedne hemokromatoze i beta-talasemije može biti dosta složen zbog činjenice da teži oblici beta-talasemije sami po sebi mogu imati za posljedicu hemokromatozu. S druge strane, i najlakši oblik beta-talasemije može dovesti do ozbiljnih manifestacija hemokromatoze u bolesnika s heterozigotnim oblikom HFE polimorfizama. Ova stanja, kao i HFE polimorfizmi naslijeđeni u homozigotnom obliku kao rizični čimbenici za razvoj hemokromatoze mogu imati za posljedicu cirozu jetre i hepatocelularni karcinom pa pravodobno prepoznavanje navedenih stanja ima presudno značenje za dužinu i kvalitetu bolesnikova života.

Metode: Za prikaz slučaja izabran je bolesnik primljen na Kliniku za zarazne bolesti Kliničke bolnice Osijek s febrilitetom, hepatosplenomegalijom i neurološkim simptomima. Kod prijma su učinjene osnovne laboratorijske pretrage te ultrazvučni pregled abdomena i snimanje glave magnetskom rezonancijom. Naknadno je provedena biopsija jetre, elektroforeza hemoglobina te određivanje koncentracije haptoglobina i utvrđivanje Cys282Tyr polimorfizma HFE gena.

Rezultati: Anamnestički podaci i rezultati rutinske laboratorijske obrade su ukazali na mogućnost da bolesnik boluje od beta-talasemije. Proširenom laboratorijskom obradom je dijagnosticirana pigmentna ciroza-hemokromatoza, a potvrđena je dijagnoza beta-talasemije minor. Odgovarajući molekularno-dijagnostički postupak je dokazao prisutnost homozigotnog oblika nasljedne hemokromatoze.

Zaključak: Određivanje koncentracije feritina i zasićenje transferina željezom, elektroforeza hemoglobina i utvrđivanje polimorfizma Cys282Tyr HFE gena pokazali su se ključnim čimbenicima za relativno brzo postavljanje ispravne dijagnoze u prikazanom slučaju istodobnog nasljeđivanja beta-talasemije minor i homozigotnog oblika nasljedne hemokromatoze. Homozigotni oblik nasljedne hemokromatoze uz beta-talasemiju minor objašnjava težinu simptoma prisutnih kod bolesnika u vrijeme hospitalizacije.

Ključne riječi: hemokromatoza; beta-talasemija; zasićenje transferina željezom; HFE; elektroforeza hemoglobina

Abstract

Background: Diagnostic approach to the simultaneous inheritance of beta-thalassemia and hereditary hemochromatosis might be quite complex due to the fact that severe beta-thalassemia itself may lead to hemochromatosis. On the other hand, beta-thalassemia minor accompanied by some heterozygous form of HFE polymorphism may also lead to the disease manifestation. These conditions as well as the homozygous forms of HFE polymorphisms are hemochromatosis risk factors that may lead to liver cirrhosis and hepatocellular carcinoma. Therefore, early diagnosis is crucial for patient quality of life and life expectancy.

Methods: A febrile patient admitted to Department of Infectious Diseases, Osijek University Hospital, with hepatosplenomegaly and some neurological symptoms has been chosen for this case report. Basic laboratory tests as well as ultrasound examination of the abdomen and magnetic resonance imaging of the head were performed shortly upon admission. Liver biopsy, hemoglobin electrophoresis, haptoglobin concentration and Cys282Tyr polymorphism determination were subsequently obtained.

Results: History data and laboratory findings suggested the diagnosis of beta-thalassemia. Extended laboratory work-up pointed to the diagnosis of pigment cirrhosis-hemochromatosis, and verified the diagnosis of beta-thalassemia minor. Appropriate molecular diagnostic procedure indicated the homozygous form of hereditary hemochromatosis.

Conclusions: In this case of homozygous hereditary hemochromatosis and beta-thalassemia minor coinheritance, serum ferritin concentration, transferrin saturation, hemoglobin electrophoresis and HFE gene Cys282Tyr polymorphism analysis proved to be crucial for the relatively fast establishment of accurate diagnosis. Recognition of the homozygous form of hemochromatosis in association with beta-thalassemia minor explained the complexity and severity of the disease presentation.

Key words: hemochromatosis; beta-thalassemia; transferrin saturation; HFE; hemoglobin electrophoresis

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Uvod

U organizmu se normalno nalazi 800-1500 mg željeza koje je uskladišteno u makrofazima u jetri, slezeni i koštanoj srži. Ako unos željeza nadmaši mogućnost njegovog fiziološkog skladištenja u navedenim organima, ono se gomila u parenhimnim stanicama različitih organa u obliku topljivog feritina, a poslije i u obliku netopljivog hemosiderina. Bolest kod koje dolazi do prekomjernog nakupljanja i toksičnog djelovanja iona željeza u jetri, srcu, gušterači, hipofizi, zglobovima i koži naziva se hemokromatozom (1).

Prema etiologiji hemokromatoze mogu biti primarne, tj. genetske i sekundarne. Genetski uvjetovanim hemokromatozama pripadaju nasljedna hemokromatoza (engl. *hereditary hemochromatosis*, HH), nasljedne hemolitične anemije i kongenitalna atransferinemija, dok se stečena hemokromatoza javlja nakon brojnih transfuzija krvi, primjene parenteralnih pripravaka ili dugotrajnog peroralnog uzimanja željeza, kod nekih oblika hepatitisa, te pijenjem pića koja se proizvode u željeznim posudama (afrička nutricijska hemokromatoza) (1).

Nasljedna hemokromatoza je autosomno-recesivna bolest. Iako učestalost manifestne HH varira ovisno o testovima probira, prosječna učestalost se kreće oko 0,5% (2). Postoji nekoliko tipova HH, a najčešći oblik je tip 1 kod kojeg dolazi do mutacije Cys282Tyr HFE gena smještenog na kratkom kraku kromosoma 6. Proteinski produkt divljeg tipa HFE gena olakšava preuzimanje plazmatskog željeza putem što ga posreduje transferinski receptor u stanicama duodenalnih kripta, dok mutirani protein HFE nema taj učinak. Funkcionalni gubitak proteina HFE uslijed mutacije smanjuje regulacijsku količinu željeza u kriptalnim stanicama i dovodi do povećane ekspresije proteina za transport željeza u enterocitima te do povećane resorpcije željeza iz hrane (2). U prosječno 83% slučajeva klinički manifestne HH riječ je o homozigotima za mutaciju Cys282Tyr (3).

Simptomi bolesti se obično javljaju u dobi nakon 40 godina u muškaraca i nakon 50 godina u žena. Kliničke manifestacije bolesti uključuju učestale infekcije, bolesti jetre, pojačanu kožnu pigmentaciju, šećernu bolest, artropatije, kardiomiopatije i impotenciju. Najčešći razlog javljanja liječniku je umor i artralgija, a većina bolesnika je asimptomatska te se otkrije samo povišena razina željeza u serumu (1).

Rano otkrivanje HH važno je za sprječavanje razvoja najtežih manifestacija, tj. ciroze jetre i hepatocelularnog karcinoma (4). U tu svrhu provodi se dijagnostička obrada koja uključuje laboratorijske analize poput određivanja koncentracije serumskog željeza (> 32 μmol/L), serumskog feritina (> 500 μg/L), zasićenja transferina željezom (> 62%), biopsiju jetre i molekularne analize polimorfizma HFE gena. Odgovarajuće referentne vrijednosti su dane u zagradama. Uz laboratorijsku obradu potrebno je učiniti ultrazvučni pregled abdomena ili primijeniti druge slikovne dijagnostičke tehnike poput kompjutorizirane tomografije ili magnetske rezonancije.

Introduction

Approximately 800-1500 mg of iron mainly deposited in the liver, spleen and bone marrow macrophages is the usual amount of this metal present in the human body. If iron intake exceeds the depot capacities of these organs, it will start to accumulate in the parenchyma of different organs in the form of soluble ferritin, and later in the form of insoluble hemosiderin. The disease characterized by iron accumulation and its toxic effects on the liver, heart, pancreas, pituitary gland, joints and skin is called hemochromatosis (1).

Based on the specific cause, hemochromatoses are divided into two groups of hereditary or genetic type and secondary type hemochromatosis. Hereditary hemochromatoses are subdivided into hereditary hemochromatosis (HH), hereditary hemolytic anemias and congenital atransferrinemia, whereas secondary hemochromatoses are subdivided into blood transfusion related type, iron therapy related type, hepatitis related type and African nutritional hemochromatosis caused by the intake of alcohol drinks prepared in iron containers (1).

Hereditary hemochromatosis is an autosomal recessive disorder. Although the prevalence of clinically manifested HH depends on the screening methods used, it has been estimated to approximately 0.5% (2). There are three genetic subtypes of HH. The most common subtype is type 1 characterized by Cys282Tyr mutation of the HFE gene located on the short arm of chromosome 6. HFE gene product is responsible for the blood iron uptake into the duodenal crypt cells. This HFE function is mediated by transferrin receptor. The described mutation disables regulation of the blood iron content and leads to enhanced expression of proteins responsible for iron transfer and increased iron absorption (2). A homozygous form of Cys282Tyr mutation has been detected in approximately 83% of all HH cases with clinical manifestations (3).

The disease symptoms usually become apparent after age 40 in male and around age 50 in female patients. Clinical manifestations of hemochromatosis include frequent infections, liver disease, intensive skin pigmentation, diabetes mellitus, arthropathies, cardiomyopathies and impotence. Most patients are asymptomatic, whereas the most common cause for visiting doctor's office are fatigue and arthralgia. In most cases, the only suspect laboratory finding is increased blood iron concentration (1).

Early recognition of HH is crucial to prevent the most severe sequels like liver cirrhosis and hepatocellular carcinoma (4). For this purpose, the following laboratory tests should be obtained: blood iron (> 32 μmol/L), ferritin concentration (> 500 μg/L), transferrin saturation (> 62%), liver biopsy and molecular analysis of known HFE polymorphisms. Appropriate cut off values are given in parentheses. Along with these tests, ultrasonography of the abdomen and other imaging methods like computerized tomography or magnetic resonance can be performed.

Cilj liječenja je sniženje koncentracije serumskog željeza i njeno održavanje unutar ili blizu referentnih vrijednosti. To se postiže venepunkcijama i/ili primjenom kelirajućih lijekova, npr. desferioksamina. Najvažniji prognostički čimbenik u vrijeme dijagnoze je prisutnost ili odsutnost fibroze ili ciroze jetre. Bolesnici liječeni u fazi prije razvoja ciroze imaju normalan medijan preživljavanja, a oni neliječeni umiru za približno 2 godine, najčešće uslijed zatajivanja srca, ciroze ili hepatocelularnog karcinoma.

Talasemije su heterogena skupina nasljednih hemolitičnih anemija uzrokovanih genetskim poremećajem u sintezi alfa- ili beta-globinskih lanaca. Podskupina beta-talasemija uključuje sindrome poremećene sinteze beta-hemoglobinskih lanaca i javlja se u tri klinička entiteta: beta-talasemija major, intermedija i minor. Kako su mutacije koje dovode do pojave ove bolesti brojne i raznolike, njihovo određivanje se ne provodi prilikom rutinske laboratorijske obrade, već se dijagnoza postavlja prema vrijednostima crvene krvne slike, elektroforeze hemoglobina i prema kliničkoj slici. Kod klinički najlakšeg oblika, beta-talasemije minor (BTM), tj. heterozigotne beta-talasemije, javlja se mikrocitoza i hipokromija eritrocita, s tek naznačenom anemijom, dok je u elektroforezi hemoglobina tek nešto povećan udio hemoglobina A2 (hemoglobin s dva alfa- i dva delta-lanca). Ostali rezultati laboratorijskih analiza su obično unutar referentnih raspona. Za ovaj oblik talasemije nije znakovita pojava hemokromatoze niti se javljaju drugi teži simptomi poput teške anemije, hemolize, koštanih deformiteta itd. koji su znakoviti za beta-talasemiju major i intermediju (2).

Uz HH i teži oblici beta-talasemije mogu samostalno dovesti do klinički značajnih simptoma hemokromatoze. Nadalje, HFE mutacije i BTM se mogu nasljeđivati i zajedno, a utvrđeno je da su u tom slučaju simptomi same hemokromatoze znatno teži (5-7). Čak i neki HFE heterozigoti koji naslijede i BTM mogu pokazivati kliničke značajne hemokromatoze (8,9). Navedene činjenice upućuju na potrebu za osvrtom na dijagnostičke postupke prikladne za prepoznavanje bolesnika koji su istodobno naslijedili obje bolesti, kao i za procjenu rizika za razvoj hemokromatoze u takvih bolesnika. U nastavku se u okviru prikaza bolesnika daje osvrt na diferencijalno-dijagnostičke postupke prikladne za utvrđivanje uzroka i težine hemokromatoze u bolesnika s Cys282Tyr HFE polimorfizmom i beta-talasemijom.

Materijali i metode

Prikaz slučaja

Bolesnik A. A. star 46 godina primljen je 2007. godine na Kliniku za zarazne bolesti Kliničke bolnice Osijek zbog febriliteta i lošeg općeg stanja. Ultrazvučnom dijagnostikom u okviru slikovne dijagnostičke obrade potvrđena je difuzno oštećenje jetre i splenomegalija. Zbog nespe-

The aim of patient treatment is to reduce blood iron concentration to normal values. It can be achieved by therapeutic phlebotomy and treatment with chelating agents like desferrioxamine. The most important prognostic factor at the time of diagnosis is the presence or absence of liver fibrosis or cirrhosis. Patients treated before the occurrence of liver cirrhosis have normal life expectancy, whereas untreated patients die in 2 years, most frequently from heart failure, cirrhosis or hepatocellular carcinoma.

Thalassemias are a heterogeneous group of hereditary hemolytic anemias caused by genetic defects that lead to inadequate synthesis of alpha- and/or beta hemoglobin chains. The beta-thalassemia subgroup includes the syndromes of defective beta-hemoglobin chain synthesis and is subdivided into three clinical types of beta-thalassemia major, intermedia and minor. Because of the large number and diversity of underlying mutations, their determination is not part of the routine laboratory work-up in beta-thalassemia patients. The diagnosis and classification of beta-thalassemia are made on the basis of complete blood count, hemoglobin electrophoresis and clinical presentation. The simplest form, beta-thalassemia minor (BTM), i.e. heterozygous beta-thalassemia, is manifested by microcytosis and hypochromia, with only slight anemia, while hemoglobin electrophoresis shows slight increase of hemoglobin A2 (hemoglobin with two alpha- and two delta-chains). Other laboratory findings usually fall within the reference ranges. Hemochromatosis, severe anemia, bone deformities and other severe manifestations compatible with beta-thalassemia major or intermedia are not characteristic of BTM (2).

Besides HH, severe forms of beta-thalassemia can also lead to hemochromatosis. Furthermore, simultaneous inheritance of some HFE mutation and BTM may also occur. In these cases, the symptoms of hemochromatosis are more pronounced (5-7). Even some of the heterozygous HFE carriers suffering from BTM may develop hemochromatosis related symptoms (8,9). All these facts point to the need of an overview of the diagnostic work-up required for timely identification of patients with coinheritance of these two diseases and of the procedures appropriate for the risk stratification for the development of hemochromatosis in these patients. Such an overview is given as part of the following case report in which we present a patient that had simultaneously inherited a homozygous Cys282Tyr HFE mutation and BTM.

Materials and methods

Case report

In 2007, A. A., a 46-year-old man, was admitted to Department of Infectious Diseases, Osijek University Hospital, for febrile condition and poor general state. Ultrasonography of the abdomen done on admission revealed a dif-

cifičnih neuroloških smetnja na koje se žalio kod prijma u smislu glavobolje i osjećaja trnjenja kože lica učinjeno je snimanje magnetskom rezonancijom te su opisane zone demijelinizacije frontalno u području bijele moždane tvari. Tijekom boravka na Klinici je prema opisanim simptomima postavljena sumnja na akutni meningoencefalitis.

Kako je anamnestički dobiven podatak da bolesnikova majka boluje od beta-talasemije, kod prijma nije provedena nikakva obrada mikrocitne anemije utvrđene kod bolesnika. U crvenoj krvnoj slici bilježe se slijedeće vrijednosti: koncentracija eritrocita 4,82 x 10^{12} /L, koncentracija hemoglobina 102 g/L, hematokrit 0,316 L/L, srednji volumen eritrocita 65,5 fL, srednji sadržaj hemoglobina 21,2 pg, srednja koncentracija hemoglobina u eritrocitima 323 g/L i koncentracija trombocita 72 x 10^9 /L. Ostalim laboratorijskim postupcima utvrđene su granične vrijednosti serumskog željeza (31,6 µmol/L), feritina (3257 µg/L) i zasićenja transferina željezom (77%) uz uredne vrijednosti jetrenih aminotransferaza (aspartat-aminotransferaza 30 U/L, alanin-aminotransferaza 25 U/L, gama-glutamiltransferaza 43 U/L). Uz vrijednosti crvene krvne slike koje su bile u skladu s beta-talasemijom, nalazi koncentracije željeza, zasićenja transferina željezom i feritina ukazali su na moguću prisutnost hemokromatoze. Kako bi se potvrdio nalaz beta-talasemije te utvrdio tip ove bolesti dodatno je učinjena elektroforeza hemoglobina i određivanje koncentracije haptoglobina. Tom je prilikom uočen povišen udio hemoglobina A2 (4,3%) uz normalnu vrijednost hemoglobina F (1,2%) i haptoglobina (0,43 g/L), što je potvrdilo dijagnozu BTM (2).

Budući da su vrijednosti serumskog željeza, feritina i zasićenja transferina željezom bile više nego što se očekuje za BTM, uz fizikalni nalaz hepatosplenomegalije učinjena je biopsija jetre. Dobivena histološka slika odgovarala je pigmentnoj cirozi, hemokromatozi. U daljnjem tijeku je radi definiranja etiologije bolesti provedena PCR-RFLP analiza HFE gena, te je dokazan homozigotni oblik polimorfizma Cys282Tyr. Kod bolesnika je provedeno liječenje desferoksaminom i venepunkcijama.

Bolesnik se nakon dvije godine liječenja subjektivno dobro osjeća. Od kontrolnih dijagnostičkih pretraga učinjen je elektrokardiogram koji je bio uredan, ultrazvuk abdomena opisuje jetru primjerene veličine te uvećanu slezenu (dimenzija oko 17 x 2 cm), bez drugih patoloških nalaza. U laboratorijskim nalazima uočava se mikrocitna hipokromna anemija (koncentracija eritrocita 5,07 x 10^{12} /L, hemoglobin 114 g/L, srednji volumen eritrocita 69,8 fL, srednji sadržaj hemoglobina 22,5 pg), porast jetrenih aminotransferaza (aspartat-aminotransferaza 81 IU/L, alanin-aminotransferaza 75 IU/L, gama-glutamil transferaza 54 U/L), HbA_{1c} graničnih vrijednosti (6,5%), te visoke vrijednosti feritina (2694 µg/L) i zasićenosti transferina željezom (70%). Vrijednosti serumskog željeza bile su unutar referentnih vrijednosti (29,2 µmol/L). Ostali laboratorijski

fuse liver lesion and splenomegaly. Magnetic resonance imaging was performed to elucidate nonspecific neurologic symptoms like headache and facial skin sensations, and revealed demyelination zones in the frontal part of the brain. Based on the symptoms, acute meningoencephalitis was suspected during his stay at Department of Infectious Diseases.

As family history data revealed the patient's mother to suffer from BTM, no detailed work-up for the patient's microcytic anemia was done. Complete blood count showed the following results: red blood cell concentration 4.82 x 10^{12} /L, hemoglobin concentration 102 g/L, hematocrit 0.316 L/L, mean cellular volume 65.5 fL, mean cellular hemoglobin content 21.2 pg, mean cellular hemoglobin concentration 323 g/L and platelet concentration 72x10^9 /L. Other laboratory findings included borderline serum iron concentration (31.6 µmol/L), significantly increased ferritin (3257 µg/L) and transferrin saturation (77%), while the levels of liver enzymes were within the reference range (aspartate aminotransferase 30 U/L, alanine aminotransferase 25 U/L, gamma-glutamyltransferase 43 U/L). Along with complete blood count values that were compatible with BTM, serum concentration of iron, ferritin and transferrin saturation pointed to the possible hemochromatosis. In order to confirm the BTM diagnosis and to determine the type of the disease, hemoglobin electrophoresis and haptoglobin concentration measurements were additionally performed to yield a slightly increased percentage of hemoglobin A2 (4.3%) and normal values of hemoglobin F (1.2%) and haptoglobin concentration (0.43 g/L); these findings confirmed the diagnosis of BTM (2).

As the values of serum iron, ferritin and transferrin saturation exceeded the values expected in BTM and hepatosplenomegaly was found, the patient underwent liver biopsy. Microscopic examination revealed pigment cirrhosis-hemochromatosis. Subsequent diagnostic work-up included PCR-RFLP analysis of HFE gene, which identified the homozygous form of Cys282Tyr polymorphism. At this point, therapeutic phlebotomy and desferrioxamine therapy were initiated.

After two years of treatment, the patient was feeling well. Ultrasonography showed enlarged spleen (approximately 17x2 cm) and the liver of regular size, while electrocardiogram revealed no abnormalities. Microcytic anemia persisted (red blood cell concentration 5.07 x 10^{12} /L, hemoglobin concentration 114 g/L, mean cellular volume 69.8 fL, mean hemoglobin content 22.5 pg), but liver enzymes activities were increased (aspartate aminotransferase 81 U/L, alanine aminotransferase 75 U/L, gamma-glutamyltransferase 54 U/L). The HbA_{1c} percentage showed borderline increase (6.5%), along with elevated ferritin (2694 µg/L) and transferrin saturation (70%). Serum iron concentration was within the reference interval

parametri se nisu značajnije mijenjali u odnosu na početne vrijednosti.

Metode

Od primijenjenih slikovnih postupaka ultrazvučna dijagnostika je provedena na instrumentu Sonoline G50 sa sondom od 3,5 MHz (Siemens AG, Erlangen, Njemačka), dok se za snimanje temeljeno na nuklearnoj magnetskoj rezonanciji rabio instrument EPIOS s jakošću polja od 0,5 T (Shimadzu Co., Kyoto, Japan). Elektrokardiogram je snimljen na uređaju PageWriter 100 (Hewlett-Packard, Palo Alto, SAD). Hematološke laboratorijske analize krvi uzete na EDTA antikoagulans su provedene na uređaju SF-3000 (Sysmex Corporation, Cobe, Japan), a sve automatizirane biokemijske analize u serumu osim niže navedenih su provedene na instrumentu Olympus AU 640 (Olympus, Hamburg, Njemačka). Za određivanje koncentracije haptoglobina u serumu primijenjen je nefelometar BN ProSpec (Siemens AG, Erlangen, Njemačka), a za određivanje HbA_{1c} u krvi uzetoj na EDTA antikoagulans primijenjen je uređaj Dimension RXL (Siemens AG, Erlangen, Njemačka). Elektroforeza hemoglobina je provedena primjenom kita Hydragel Hemoglobin K20 proizvođača Sebia, Inc., Norcross, GA, USA, a histopatološka analiza uzorka dobivenog biopsijom jetre provedena je uz bojenje preparata berlinskim modrilom. Polimorfizam Cys282Tyr HFE gena je utvrđen metodom PCR-RFLP opisanom u literaturi (3).

Rasprava

Istodobno nasljeđivanje beta-talasemije i različitih mutacija HFE gena je dosad nekoliko puta opisano u literaturi (5-9). Ono što slučaj bolesnika A. A. čini posebnim je neuobičajena pojavnost odnosno težina simptoma, poput ciroze i demijelinizacije te činjenica da je uz beta-talasemiju minor utvrđena homozigotna varijanta Cys282Tyr HFE polimorfizma. U literaturi su se dosad uglavnom obrađivali slučajevi istodobnog pojavljivanja beta-talasemije i heterozigotnih varijanta HFE polimorfizama.

Bolesnik je prilikom prvog pregleda bio bez jasnih kliničkih simptoma koji bi ukazali na moguću hemokromatozu. Štoviše, vodeći su simptomi bili osnova za sumnju na akutni meningoencefalitis. Aktivnosti jetrenih aminotransferaza su bile uredne, što je otežalo dijagnostičku obradu. Od iznimnog značenja se u obradi bolesnika A. A. pokazalo određivanje koncentracije feritina i zasićenosti transferina željezom. Naime, povećane vrijednosti ovih dvaju parametara koji su dio proširene laboratorijske obrade prve su ukazale na hemokromatozu.

Isprva nije bilo jasno značenje beta-talasemije za bolesnikovo stanje preopterećenosti željezom, koja je na osnovi anamneze i rutinskih laboratorijskih nalaza pripisana ovom bolesniku. Iako beta-talasemija može dovesti do pojave hemokromatoze, tek najteži oblici beta-talasemi-

(29.2 µmol/L). Other laboratory parameters did not change significantly in comparison to their initial values.

Methods

The imaging techniques employed were ultrasonography, which was carried out on a Sonoline device with 3.5 MHz G50 probe (Siemens AG, Erlangen, Germany), and magnetic resonance imaging, which was carried out on an EPIOS device with magnetic field strength of 0.5 T (Shimadzu Co., Kyoto, Japan). Electrocardiogram was recorded on a PageWriter 100 instrument (Hewlett-Packard, Palo Alto, USA). Complete blood count was determined on an SF-3000 counter (Sysmex Corporation, Cobe, Japan) and EDTA anticoagulant was used. With the exception of automated biochemical analyses described below, all other analyses from this group were performed on an AU 640 device (Olympus, Hamburg, Germany). A BN ProSpec nephelometer (Siemens AG, Erlangen, Germany) was used for haptoglobin determination, and a Dimension RXL device (Siemens AG, Erlangen, Germany) for determination of HbA_{1c} percentage in whole blood taken on EDTA anticoagulant. Hemoglobin electrophoresis was performed by the application of Hydragel Hemoglobin K20 kit (Sebia, Inc., Norcross, GA, USA), while histopathology of the liver biopsy sample was performed after tissue processing by Prussian blue dye. HFE gene Cys282Tyr polymorphism was analyzed by the PCR-RFLP procedure described in the literature (3).

Discussion

There are several literature reports on simultaneous inheritance of beta-thalassemia and different HFE mutations (5-9). Unusual presentation of these diseases in the patient presented and severe symptoms like cirrhosis and demyelination caused by the homogeneous form of Cys282Tyr HFE mutation coinherited with BTM makes this case worthy of closer examination. In most cases described in the literature, patients suffered from heterozygous types of HFE mutations coinherited with beta-thalassemia. On admission, the patient did not exhibit any overt sign of hemochromatosis. On the contrary, the leading symptoms were suggestive of diagnostic procedures for acute meningoencephalitis. Liver enzyme activities were within the reference intervals and this finding made the diagnostic work-up even more complex. Highly increased values of ferritin and transferrin saturation proved to be of key importance. These two parameters of extended laboratory work-up were the first to show the underlying hemochromatosis.

Suspicion of beta-thalassemia was indicated in the patient's history and the findings of complete blood count were compatible with this notion. At first, the significance of this disease for the etiology of hemochromatosis was

je ili HH mogu dovesti do funkcionalnog hiposplenizma koji se očituje učestalim infekcijama, te do morfološke splenomegalije, što je bio slučaj kod bolesnika A. A. (2). U bolesnika s hemokromatozom se rijetko mogu javiti i neurološki simptomi (10,11), što je također bio slučaj kod ovog bolesnika. No, i takve manifestacije hemokromatoze su spojive samo s težim oblicima beta-talasemije ili HH. Stoga je od presudne važnosti bilo utvrditi tip beta-talasemije. Tek je nakon učinjene elektroforeze hemoglobina postalo jasno da dijagnosticirana beta-talasemija minor samostalno ne može biti uzrokom manifestne hemokromatoze u ovog bolesnika.

Iz prikazanog slučaja se može uočiti da se kod našeg bolesnika već bila razvila pigmentna ciroza jetre, što se smatra jednom od najtežih posljedica neliječene HH. Prisutnost hemokromatoze i ciroze jetre utvrđene biopsijom, te BTM ukazuje na to da je najvjerojatnije riječ o nasljednoj mutaciji HFE gena. Da je doista riječ o homozigotnom obliku HH potvrđeno je molekularnom dijagnostikom. Tek postavljanje dijagnoze HH i BTM nedvojbeno razjašnjava relativno teške i neuobičajene simptome hemokromatoze poput ciroze jetre i demijelinizacije.

Rezultati dijagnostičke obrade dobiveni nakon dvije godine terapije pokazuju neznatno poboljšanje metabolizma željeza. Nažalost, uočava se istodobni porast aktivnosti jetrenih aminotransferaza te granično oštećena funkcija endokrine gušterače. Na temelju navedenih rezultata promjena terapije koja bi trebala ići bilo u smjeru češćih venepunkcija bilo u smjeru promjene doze ili vrste kelatora željeza čini se nužnom. Kako je riječ o dvije nasljedne bolesti koje pod određenim uvjetima mogu dovesti do manifestne hemokromatoze, potrebno je provesti i pregled članova bolesnikove obitelji radi sprječavanja razvoja bolesti.

Zaključak

Iako je istodobno nasljeđivanje dviju bolesti malo vjerojatno, ono se ne smije isključiti iz dijagnostičkog postupka, što opisani slučaj potvrđuje. Kao ključne čimbenike za postavljanje ispravne dijagnoze u prikazanom slučaju treba navesti određivanje koncentracije feritina i zasićenja transferina željezom, elektroforezu hemoglobina i utvrđivanje polimorfizma Cys282Tyr HFE gena. Bez navedenih laboratorijskih analiza, osobito određivanja koncentracije feritina i zasićenja transferina željezom, postavljanje sumnje na hemokromatozu kao etiološkog čimbenika za pojavu neuobičajenih simptoma zbog kojih je bolesnik hospitaliziran bilo bi značajno odgođeno.

Prikazani slučaj nadalje pokazuje kako se beta-talasemiji kao etiološkom čimbeniku za pojavu hemokromatoze treba pristupiti izrazito oprezno. Samo liječenje beta-talasemije ne može smanjiti simptome zbog kojih je bolesnik hospitaliziran. S druge strane, BTM može dovesti do

not clear. Although beta-thalassemia could lead to hemochromatosis, only a severe form of beta-thalassemia or HH could lead to functional hyposplenism, along with morphological hypersplenism that presents in the form of frequent infections, as was the case in our patient (2). Although rarely, the neurologic symptoms recorded in our patient may also manifest in hemochromatosis patients (10,11). These manifestations are only compatible with the severe forms of beta-thalassemia or HH. Therefore, it was of essential to determine the type of beta-thalassemia. Only after hemoglobin electrophoresis it was evident that beta-thalassemia minor alone could not have caused such symptoms.

As mentioned above, the patient had already developed liver cirrhosis as one of the most severe hemochromatosis symptoms. Hemochromatosis and liver cirrhosis verified by biopsy along with BTM pointed to the most probable cause of the patient's illness, i.e. some kind of HFE polymorphism. The homozygous form of HH was confirmed by molecular diagnosis. It was only the diagnosis of coinherited BTM and homozygous HH that could accurately explain the relatively severe and unusual symptoms of hemochromatosis such as cirrhosis and demyelination.

The results obtained at two years of therapy introduction showed only minor improvement of iron metabolism. Unfortunately, there was a simultaneous increase in the liver enzyme activities and borderline functional impairment of endocrine pancreas. Based on these results, it is concluded that there therapy should be modified, either by the introduction of more frequent therapeutic phlebotomy or change in the dosage or type of iron chelator therapy. Since the patient suffers from two diseases which, under certain conditions, can lead to hemochromatosis manifestations, it is necessary to examine his family members in order to prevent the disease development.

Conclusion

Although simultaneous inheritance of two diseases is not very likely, the case presented shows that this scenario should not be overlooked on diagnostic work-up. In the case presented, serum ferritin, transferrin saturation, hemoglobin electrophoresis and Cys282Tyr HFE polymorphism analysis proved to be the key factors to reach the accurate diagnosis. Without these laboratory results, serum ferritin and transferrin saturation in particular, the recognition of hemochromatosis as a possible underlying cause of unusual symptoms that led to the patient's hospitalization, would have been considerably delayed.

The case presented shows that beta-thalassemia should be carefully examined as a possible cause of hemochromatosis development. The treatment for beta-thalassemia would not alleviate the symptoms that require hospitalization. On the other hand, BTM could lead to

manifestne hemokromatoze čak i u heterozigota za HFE polimorfizme. Činjenica da je bolesnik A. A. uz BTM homozigot za Cys282Tyr HFE mutaciju može objasniti težinu simptoma koji su uočeni prilikom njegove prve hospitalizacije.

Konačno, kako je asimptomatski tijek HH vrlo dug, njezino rano otkrivanje značajno produžuje život bolesnika te poboljšava njegovu kvalitetu. Budući da se HH i beta-talasemija mogu relativno lako prepoznati na temelju jednostavnih i lako dostupnih laboratorijskih pretraga, od osobitog je značenja njihovo praćenje u obiteljima oboljelih u svrhu otkrivanja nositelja ili asimptomatskih članova. Zbog raznolike kliničke slike i složenih međuodnosa mogućih uzroka hemokromatoze potrebna je pažljiva interpretacija dobivenih rezultata.

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hemochromatosis manifestation even in heterozygous HFE mutation carriers. The fact that the patient presented suffered from both BTM and the homozygous form of HH explains the unusually severe hemochromatosis presentation at the time of diagnosis.

Finally, due to the long period of latency, early recognition of HH improves the patient's chances for normal life expectancy and quality. The fact that simple and widely available laboratory tests could accurately reveal HH and beta-thalassemia justifies their use in the screening of patient families for carriers and asymptomatic members. Due to the diverse presentation and complex interplay of the causes of hemochromatosis in these patients, only careful interpretation of the results obtained can ensure establishment of accurate diagnosis and treatment.

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Literatura/References

1. *Fauci SA, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J, eds. Harrison's Principles of Internal Medicine. 17th ed. New York: McGraw-Hill, 2008.*
2. *McKenzie SB, ed. Clinical Laboratory Hematology. Prentice Hall, 2006.*
3. *Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399-408.*
4. *Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology 2004;127(5 Suppl 1):S79-86.*
5. *Martins R, Picanço I, Fonseca A, Ferreira L, Rodrigues O, Coelho M, et al. The role of HFE mutations on iron metabolism in beta-thalassemia carriers. J Hum Genet 2004;49:651-5.*
6. *Oliveira TM, Souza FP, Jardim AC, Cordeiro JA, Pinho JR, Sitnik R, et al. HFE gene mutations in Brazilian thalassemic patients. Braz J Med Biol Res 2006;39:1575-80.*
7. *Piperno A, Mariani R, Arosio C, Vergani A, Bosio S, Fargion S, et al. Haemochromatosis in patients with beta-thalassaemia trait. Br J Haematol 2000;111:908-14.*
8. *Ruiz-Argüelles GJ, Garcés-Eisele J, Reyes-Núñez V, Sánchez-Anzaldo J, Ruiz-Delgado GJ, Jiménez-González C, Carrera B. Heterozygosity for the H63D mutation in the hereditary hemochromatosis (HFE) gene may lead into severe iron overload in beta-thalassemia minor: observations in a thalassemic kindred. Rev Invest Clin 2001;53:117-20.*
9. *Arruda VR, Agostinho MF, Cançado R, Costa FF, Saad ST. Beta-thalassemia trait might increase the severity of hemochromatosis in subjects with the C282Y mutation in the HFE gene. Am J Hematol 2000;63:230.*
10. *Misawa S, Kuwabara S, Matsuda S, Sakakibara Y, Ogawa Y, Tashiro Y, Hattori T. Chronic inflammatory demyelinating polyneuropathy associated with idiopathic hemochromatosis. Intern Med 2006;45:871-3.*
11. *Demarquay G, Setiey A, Morel Y, Trepo C, Chazot G, Broussolle E. Clinical report of three patients with hereditary hemochromatosis and movement disorders. Mov Disord 2000;15:1204-9.*