tate modificări importante, manifestate prin conţinut seric crescut al IgM (3,06±0,14 g/l); p<0,001) şi IgG (28,78±2,12 g/l; p<0,001) (*tabelul 3*), versus valorile respective din lotul-martor. Totodată, la pacienţii lotului de studiu se atestă valori crescute ale complexurilor imunocirculante – 315,72±55,31 UDO.

Tabelul 3

Indicii imunităţii umorale la pacienţii cu HAI

Indici	*Lotul-martor – I (n=20)*	*HAI – II (n=20)*	p_{I-II}
Ig A (g/l)	2,45±0,10	2,96±0,22	p>0,05
Ig M (g/l)	0,81±0,04	3,06±0,14	P<0,001
Ig G (g/l)	10,66±0,31	28,78±2,12	p<0,001
CIC (UDO)	94±8,36	315,72±55,31	p<0,001

În HAI, nivelul IgM, IgG şi nivelul CIC au fost veridic statistic mai majorate versus lotul-martor (p<0,001), ceea ce pledează în favoarea faptului că hepatita autoimună este însoţită de modificări imunologice semnificative.

Tratamentul bolii autoimune a ficatului prevede medicaţie specifică şi este complicat atât datorită particularităţilor individuale, cât şi din cauza dificultăţilor de diagnostic. În prezent continuă cercetările eficacităţii terapeutice a diferitelor preparate în terapia ficatului autoimun. Noi am studiat eficacitatea medicaţiei cu prednisolon în terapia pacienţilor cu hepatită autoimună. Cercetând bolnavii înainte de administratrea tratamentului şi pe parcursul terapiei imunosupresive, am constatat o evoluţie progresiv-pozitivă a sindroamelor clinice şi biologice, astfel rezultatele noastre au demonstrat efectul favorabil al prednisolonului la pacienţii cu hepatită autoimună.

Concluzii

1. Evaluarea clinică a pacienţilor cu hepatită autoimună a constatat prezenţa mai frecventă a sindroamelor astenovegetativ (100%), dispeptic (75%), dolor abdominal (60%), a hepatomegaliei (70%), splenomegaliei (65%), icterului (35%) şi sindromului articular (25%).

2. Terapia imunosupresivă a influenţat benefic evoluţia sindroamelor clinice la pacienţii cu HAI prin regresarea veridică a sindroamelor astenovegetativ, dolor, a splenomegaliei, sindromului articular şi icterului.

3. La pacienţii cu HAI, iniţial s-a determinat sindrom citolitic manifestat prin valori crescute de 5,7 ori ale ALT, versus grupul de control (p<0,01), şi de 6,7 ori mai ridicate ale AST versus lotul-martor (p<0,01). La pacienţii cu hepatită autoimună, pe parcursul tratamentului cu prednisolon s-a constatat micşorarea veridică a ALT şi AST, comparativ cu valorile iniţiale (p<0,05).

4. Iniţial, evaluarea indicilor ce reflectă sindromul hepatopriv la pacienţii cu HAI a relevat reducerea semnificativă a albuminelor, proteinei totale (p<0,01) şi a protrombinei (p<0,05), comparativ cu lotul-martor, şi a demonstrat creşterea veridică a acestor parametri pe parcursul tratamentului imunosupresor.

5. Modificările imunologice umorale se caracterizează prin creşterea predominantă a IgG şi CIC la bolnavii cu hepatită autoimună, comparativ cu persoanele sănătoase.

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PHENOTYPIC OR GENOTYPIC DIAGNOSIS OF HEMOCHROMATOSIS?

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Rezumat

Diagnosticul fenotipic sau genotipic al hemocromatozei?

Hemocromatoza ereditară (HE) este o afecţiune genetică determinată de supraîncărcarea cu fier, bazată pe mutaţia genei HFE, clasificată în 4 genotipuri, cel mai răspândit fiind genotipul I (90%). Implicarea ficatului este cea mai frecventă abnormalitate în HE, iar studiile dovedesc că indicele hepatic al fierului poate fi în limitele normei la persoanele cu HE, totodată fiind sporit la cei cu sindroame

secundare de supraîncărcare cu fier. În această publicaţie propunem spre discuţie care, totuşi, ar fi cele mai eficiente metode: genotipice ori fenotipice în stabilirea HE, bazându-se pe un caz clinic real.

***Cuvinte-cheie**: hemocromatoză, diagnostic*

Резюме

Фенотипичное и генотипичное определение гемохроматоза

Наследственный гемохроматоз – аутосомно-рецессивное заболевание, частота распространенности которого 1 человек на 200, относится к заболеваниям, при которых относительно несложно выявить генетический дефект (HFE-гена). Несмотря на полиморфность гемохроматоза, поражение печени встречается в 100% случаев, которое может регистрироваться как при случайном обследовании по поводу повышения уровня трансаминаз, так и при дебюте портальной гипертензии на фоне уже сформировавшегося цирроза печени. До генетического тестирования существует вполне пригодный метод обследования населения путем чувствительного фенотипического скрининга: определения насыщения трансферрина, количество сывороточного железа, уровень ферритина.

***Ключевые слова**: гемохроматоз, диагностика*

Introduction

HH includes a variety of chronic iron overload syndromes of genetic origin. They can be classified into 4 groups from type 1 to type 4 according to genetic mutations [1, 2]. HH is the most genetic condition known in people of European descent. It is estimated that one in 200 people with European ancestry have the typical genetic pattern associate with this condition [6, 7]. If the disease is defined based on symptoms, the prevalence would be much lower, and because the symptoms may be nonspecific, it is more difficult to assess the prevalence of symptomatic hemochromatosis [3, 8].

Classic HH is an autosomal recessive iron-overload disorder associated with mutation of the HFE gene, which is located on chromosome 6; in most cases the mutation is a single-base change that results in the substitution of tyrosine for cysteine at position 282 of the HFE protein (C282Y) [1, 2, 7]. Homozygosity for the C282Y mutation is now found in approximately 5 of every 1000 persons of northern European descent — a prevalence 10 times that of cystic fibrosis genotypes [3,5]. All persons who are homozygous for the C282Y mutation are genetically predisposed to a chain of events that may culminate in severe damage to multiple organs, but it is currently impossible to predict whether, and to what extent, the mutation will be phenotypically expressed [4, 7].

Most of the debate regarding hemochromatosis centers on its phenotypic or genotypic diagnosis. The genetic defect, which caused no serious obstacle to reproduction and may phenotypic and genetic features have conferred some advantages, was passed on and spread by population migration [7, 8].

The genetic aspect of hemochromatosis

Human iron homeostasis depends on the coordinated functions of numerous genes, the precise roles of which are, in many cases, still obscure. The major-histocompatibility- complex class I–like protein, HFE, which has an ancestral peptide-binding groove that is too narrow for antigen presentation, is also incapable of binding iron [5, 6, 8]. Newly synthesized HFE binds to beta 2 - macroglobulin, an event necessary for its expression on the cell surface and endosome membranes, where it interacts with transferrin receptor 1 (TfR1), the major receptor for transferrin. By disrupting a disulfide bond in HFE that is critical for its binding to beta 2 -macroglobulin, the C282Y mutation impairs cell-surface expression of HFE and the interaction of HFE with TfR1.

TfR2 (transferrin receptor type 2) differs from TfR1 in its affinity in vitro for transferrin (1/25 to 1/30 as strong), its high level of expression on hepatocytes, and the fact that its expression is not down- regulated by hepatic iron overload [4, 7]. Hepcidin is synthesized by hepatocytes in response to both inflammatory stimuli and iron overload. It has been hailed as "the iron-regulatory hormone," although the mechanisms underlying its effects are unclear. Other mutation in HFE have also describes, for example of a mutation in which aspartic acid replace histidine at position 63 (H63D), or cysteine replaces serine at position 65 (S65C) [1, 3, 6].

The phenotypic aspect of hemochromatosis

Whether a clinically asymptomatic patient with elevated iron levels should be classified as having HH is currently an issue of debate. Some investigators have proposed a case definition for HH comprising a sustained elevation in serum ferritin levels (>200 µg/l in women and >300 µg/l in men) and transferrin saturation (>45% in women and >50% in men) in the absence of any other risk factors for iron overload [4, 6]. This approach has the advantage of simplicity, but it would probably identify a number of patients who do not have iron overload. Other researchers insist that iron overload should be documented and quantified by liver biopsy [1, 2]. The pattern of iron distribution might be helpful in defining the cause of disease. The hepatic iron index (hepatic iron concentration divided by patient age) was devised before genetic testing, and originally used to differentiate patients with alcoholic siderosis from those with HH. Subsequent studies using genetic testing, however, have shown

that many patients with early hemochromatosis do not have an elevated hepatic iron index (>1.9), and that patients with other types of iron overload do have an elevated hepatic iron index [5, 6].

The patients with HH should have a constellation of clinical signs and symptoms. The association of any symptoms of HH with elevated iron levels or C282Y homozygosity has, however, come under increased scrutiny. Patients with bronze diabetes probably represent <1% of all HH patients, and studies have shown that the prevalence of diabetes is similar in both C282Y homozygous and control populations. Other symptoms associated with HH, such as fatigue and arthralgia, are nonspecific and very common in the general population [6, 7, 8].

Table 1

Methods for iron overload assessment

	Methods for iron overload assessment according to the type of evaluation
A	Clinical features: signs and symptoms
B	Serum markers: iron; ferritin; transferrin, transferrin saturation
C	Tissue iron concentration: liver biopsy
D	Iron toxicity markers: non transferrin bound iron, labile plasma iron; markers of oxidative damage; liver fibrosis
E	Iron balance calculation: iron load with transfusions; iron removal by phlebotomy; iron excretion by chelators.

Liver disease in HH

Thanks to increasingly early diagnosis, the classic triad of cirrhosis, bronze skin and diabetes is now rare in adult-onset HH [2, 3]. The most common symptoms at presentation in middle-aged adults are now *fatigue, malaise, arthralgia* sometimes associated with *hepatomegaly or slightly increased aminotransferase levels.* In addition, patients commonly present with increased transferrin-saturation values, which are sometimes found even in the absence of symptoms [4, 5]. Increasing serum ferritin levels herald iron accumulation in the tissues, and values above 1000 ng/ml may indicate underlying liver cirrhosis in persons homozygous for the C282Y mutation, regardless of their age or serum liver-enzyme levels [6, 7].

In the early stages of HH iron is located inside the hepatocytes in the biliary pole of the cell. Over time the iron overload increases leading to peroxidation of iron dependent lipids causing damage and periportal hepatocellular death (siderotic necrosis). Cirrhosis develops when the hepatic concentration surpasses 400 mmol/g [3, 8]. Of all of the putative symptoms of HH, liver disease is the most consistently identified abnormality. Many patients with liver disease are, however, asymptomatic—even those with hepatic fibrosis. *I believe that I'd better describe the involvement of liver in HH by presenting a real clinical case*.

Table 2

Case with hereditary hemochromatosis

CLINICAL CASE
A 45 year old male patient came to the emergency service after three days of melena and massive hematemesis. He had a record of diabetes mellitus treated with insulin for 6 years with periodic check-ups. Patient was admitted in generally bad condition with generalized mucocutaneous paleness, but patient was conscious and alert. Blood Pressure 90/60; HR 96/min; RR 20/min; Cardiopulmonary assessment: Satisfactory; Abdomen: was found by percussion – hepatospleenomegaly; no ascites and collaterals. Extremities: No edema. The patient was initially medicated with crystalloid and given a transfusion of 3 units of packed red blood cells (PRBCs). Omeprazole infusions achieved hemodynamic stability. *Personal Medical Record: Patient was hospitalized for chronic hepatitis (viral markers for hepatitis A, B, C was negative) two years prior to admission.* *Family Medical Record: Father and paternal uncle died of cirrhosis before they were 50 years old. Brother and sisters are reported to be healthy.* *Epidemiologic Records: occupation - mason. No contact with toxics, no transfusions, no acute hepatitis, occasional smoker.* *Examinations: anemia (Hb 78 g/l), prothrombin 55, INR 2.36, hyperglycemia (8.7 mmol/l), decresed total proteins (59 g/l), hypoalbuminemia (24 g/l), increased activity of ALT (96 U/L) and AST (74 U/L), alkaline phosphatase, bilirubin and gamma GT was normal. Autoimmune markers: ANA AMA, antiDNA – negative; Serological viral markers: HBsAg, anti HBs and antiHBcor total - negative, anti-HAIgM negative, anti HVC IgM – negative; anti HEV IgM – negative; He had normal serum ceruloplasmin and serum copper levels, urine copper of 18 ng/ml.* ***Serum iron****: 187 mg/dl (59 – 158);* ***Ferritin****: 1144 ng/dl (9 – 120);* ***Saturation percentage of transferrin*** *96.3% (12 – 36).* *A high* ***digestive endoscopy*** *was performed with a finding of esophageal varices. Band ligation (using #3 bands) was then performed starting from the cardia.* *A* ***sonogram*** *showed hepatospleenomegaly. The gall bladder, intrahepatic and extra hepatic bile ducts, aorta and cava were all normal.* ***Fibrotest*** *showed fibrosis F4 (confirmed cirrhosis).* **A preliminary diagnosis:** ***Liver cirrhosis secondary hereditary hemochromatosis?*** *The patient has participated in a screening study, and was found to have the genes for hemochromatosis (C282Y homozygote).* **The definitive diagnosis:** ***Hereditary hemochromatosis (homozygote by C282Y HFE) with liver cirrhosis, Child-Pugh B (9 p.).***

Management of HH. Literature review and discussion our case

HH is perceived be a rare condition, reflected in the estimate that only about one in 10000 people are diagnosed with the condition [1, 2, 9]. In reality, HH is the most genetic condition known in people of European descent. It is estimated that one in 200 people with European ancestry have the typical genetic pattern associate with this condition [3, 4]. The discrepancy in the rates of diagnosis versus prevalence of positive gene tests for HH can be explained by two causes. First, not all people who are C282Y

homozygotes go on to develop evidence of iron overload. It is estimated that 50% of these women and 50–90% of this men will express the genes as evidenced by high serum iron tests. Second, as the symptoms are nonspecific, many cases of HH may go undiagnosed.

To suspect HH when have: elevation in serum ferritin levels (>200 µg/l in women and >300 µg/l in men) and transferrin saturation (>45% in women and >50% in men) in the absence of any other risk factors for iron overload [5, 8, 9].

Case: Abnormally high levels of ferritin were found in our patient, while the transferrin level was found to be at the point of saturation. A preliminary diagnosis of HH was established. This patient does not need a liver biopsy for diagnostic purposes, as the gene test has already revealed that he has HH. Liver biopsy is currently most important for the diagnosis non-HFE related iron overload in patients with concomitant risk factors. Presence of cirrhosis in our patient decreases life expectancy, and increase risk of hepatocellular carcinoma about 200-fold as compared to normal subjects. In different series 25% to 50% of those diagnosed with HH are diagnosed when they have cirrhosis. This demonstrate the absence of specific symptoms and signals that would cause suspicion of the disease and a diagnosis of the disease, and presented case reflected this situation.

Treatment

Patients with HH should undergo therapeutic phlebotomy weekly (as tolerated). Target levels of phlebotomy should be a ferritin level of 50-100 µg/L [4, 6, 8]. In the absence of indicators suggestive of significant liver disease (ALT, AST elevation), C282Y homozygotes who have an elevated ferritin (but <1000 µg/L) should proceed to phlebotomy without a liver biopsy. Patients with end-organ damage due to iron overload should undergo regular phlebotomy to the same endpoints as indicated above. Phlebotomy has been found to be highly effective therapy for HH, preventing morbidity while promoting normal longevity. Symptoms may improve differentially during treatment. The strongest supporting evidence for a beneficial effect of phlebotomy is the improvement of liver fibrosis that has been demonstrated on serial liver biopsies in hemochromatosis patients [3, 6, 7].

Maintenance therapy is even less established following iron depletion, and many patients will not demonstrate any evidence of iron accumulation after many years of observation. Many patients enjoy the concept of continuous therapy for hemochromatosis and these patients can be encouraged to be voluntary blood donors several times per year. If they are ineligible, an annual ferritin determination is a reasonable alternative to guide maintenance therapy [2, 4, 5].

Case: Which clotting time corrected, our patient were managed with biweekly phlebotomies until levels of Hb 100-120 g/l.

Iron chelation (Deferoxamine, Deferiprone) are the prevention of iron-related complications, the maintenance of safe tissue iron levels and the reversal of iron-related complications [7, 8]. Hemochromatosis is a common and relatively simple genetic disease to diagnose and treat. It can be diagnosed and treated by family physicians using transferrin saturation, serum ferritin and C282Y genetic testing.

Conclusion

HH is a common genetic disease leading to accumulation of iron in the body, most notably in the liver. Many questions remain regarding the optimal screening strategy for HHC, the need for liver biopsy, the role of HFE genotyping, and the relationship between HFE genotype and clinical measures of iron stores. The prevalence of phenotypic HH, the excess mortality of liver cirrhosis, the quality of life in non-cirrhotic HH patients, and the fractions of patients compliant with treatment were the most important variables in the sensitivity analysis.

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