NEW MOLECULAR APPROACHES TO EARLY DETECTION AND DIAGNOSIS OF HFE-RELATED HEMOCHROMATOSIS

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HFE-related hereditary hemochromatosis (HFE-HH) is an autosomal recessive disorder of iron metabolism with a prevalence of about 5 in 1000 individuals of Caucasian descent. The massive iron deposition in parenchymal cells leads to multiorgan dysfunction. Despite its high prevalence, the illness is often undiagnosed or misdiagnosed when classical diagnostic methods are used. The life expectancy of the patients is effectively improved by a simple treatment (phlebotomy) if it is instituted before organ damage had occurred. The identification of a hemochromatosis gene (HFE), in 1996, made possible a molecular diagnosis for this disease. In multiple population studies, 85–90% of HFE-HH patients were found to be homozygous for a G to A missense mutation (C282Y) in the HFE gene (exon 4). An efficient large-scale screening method for the C282Y mutation has been applied in order to confirm the diagnosis of HFE-HH in suspected cases. This test is based on allele specific PCR (AS-PCR) combined with facilitated DNA extraction from whole blood. We report on 11 suspected cases of HFE-HH based on clinical and laboratory features. After HFE genotyping, 2 patients (18%) were found to be homozygous (C282Y/C282Y), 2 patients (18%) were heterozygous (C282Y/wild-type) and 7 subjects (64%) had a normal genotype (wild-type/wild-type). The genetic test’s results allowed, in the above cases, the proper guidance of the therapy. The knowledge gained from this study will be used to improve the HFE-HH screening methods, to assess the incidence and prevalence of HFE-HH in Romania and to decrease the morbidity and mortality in this disease.

Key words: iron overload, hemochromatosis, HFE gene mutation, screening.

INTRODUCTION

HFE-related hereditary hemochromatosis (HFE-HH) is an autosomal recessive disorder characterized by increased gastrointestinal iron absorption along with high iron release from reticuloendothelial macrophages (1, 2). Iron promotes the generation of free radicals, which leads to mutagenesis, atherosclerosis, inflammation and bacterial growth. Thus, massive iron deposition in parenchymal
cells may lead to the progressive development of life threatening complications: cirrhosis, hepatocellular carcinoma, diabetes, infertility and heart disease (3).

It is widely accepted that classical diagnostic methods are not able to correctly identify HFE-HH, whereas its prevalence in European population ranges from 0.3 to 0.5% (4). The early detection of iron overload allows a correct diagnosis of HFE-HH and facilitates the treatment, by means of therapeutic phlebotomy, slowing down the course of the disease (5–7). The biochemical diagnosis is made by measuring serum transferrin saturation and ferritin level, but it could be too late if these values are increased because too much iron has been accumulated, leading to irreversible organ damage. From the identification of HFE gene (locus 6p21.3, close to HLA-A region) in 1996 (8), a major breakthrough has been made in the diagnostic approach of this disease with the description of a G to A missense mutation (C282Y) found in more than 80% of patients diagnosed with iron overload due to HFE-HH. Genetic screening for C282Y mutation is therefore essential when iron overload is investigated (9).

Our study is first motivated by the lack of data regarding the incidence and prevalence of HFE-HH in Romania. Our purpose is twofold: to identify cases of suspected hemochromatosis by means of classical diagnostic tools and to confirm the HFE-HH affected patients among suspected cases, using genetic testing for the C282Y mutation. Until now, the liver biopsy has been the golden standard diagnostic method for these patients in Romania. The long term goal of our study is the assessment of the incidence and prevalence of HFE-HH in Romania.

MATERIAL AND METHODS

Patients. The inclusion criteria for candidates in our study were: clinical expression of HFE-HH, elevated transferrin saturation and/or ferritin level, but diagnosis of HFE-HH has not yet confirmed, by genetic test or liver biopsy, family member screening. The exclusion criteria were: age less than 18 years, patients with iron overload not due to HFE-HH (e.g., porphyria cutanea tarda, hepatitis C infection, Wilson’s disease, alpha-1-antitrypsin deficiency, alcohol abuse). Candidates had a history, physical evaluation, review of medical records and laboratory tests and filled in an 22-item symptoms questionnaire (10).

We have developed the first website (http://www.umft.ro/hemochromatoza/hemo_en/) about HFE-HH in Romania, available from February 2004 and addressed to both physicians and people not involved in health-care services. The web-based informatics system, as an interactive means of communication and education, provides the first step in the population screening.

Determination of C282Y mutation. We have applied an efficient large-scale screening method for the C282Y mutation in order to confirm the diagnosis of HFE-HH in
suspected cases. Genomic DNA was extracted from whole blood using QIAamp Blood Mini Kit (QIAGEN). C282Y mutation was detected by allele-specific PCR (AS-PCR) using four different oligonucleotide primers consisting of two non-allele-specific primers (F1: AAGCAGCCAATGGATGCCAAG and R1: CCACCTGATGACTCAATGACTA) and two allele-specific primers (Rw: CCTGGGTGCTCCACCCTGTC, Fm: GGGAGAGCGAGATATACGTA) (11). The PCR products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining under UV light. For each amplification product, one of the following genotypes was observed: C282Y/C282Y, C282Y/wild-type (C282/WT), or wild-type/wild-type (WT/WT).

RESULTS

We have tested for the C282Y mutation 11 possible cases of HFE-HH: 2 women and 9 men with a median age of 45 years (range 27–70). After HFE genotyping, 2 patients (18%) were found to be homozygous (C282Y/C282Y), 2 patients (18%) were heterozygous (C282Y/WT) and 7 subjects (64%) had a normal genotype (WT/WT) (Figure 1).

![Fig. 1. – Agarose gel electrophoresis of AS-PCR products. M = pGEM DNA marker (sizes of visualized bands are: 126, 179, 222, 350, 396, 460, 517, 676, 1198, 1605, 2645 bp).](image)

The referral criteria along with the results for the genetic testing are shown in Table 1.
Table 1

Referral criteria of the patients and results of the genetic test

<table>
<thead>
<tr>
<th>Referral criteria</th>
<th>Patients</th>
<th></th>
<th></th>
<th>Normal genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>Homozygotes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Studied (n, %)</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>– HFE-HH symptoms; – sideraemia &gt;200 µg/dl</td>
<td>8 (72.7)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>– infertility; – full-blown laboratory findings (raised transferrin saturation and ferritin concentration)</td>
<td>1 (9.1)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>– HFE-HH symptoms; – full-blown laboratory findings (raised transferrin saturation and ferritin concentration)</td>
<td>2 (18.2)</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: M = male, F = female.

DISCUSSION

HFE-HH is more common than cystic fibrosis, muscular dystrophy and phenylketonuria combined (12). To address this disease it is important to consider the continuous improvement of the diagnostic methods because the early identification of the HFE-HH is critical for the disease management and patient outcomes. A simple treatment (phlebotomy) is available and effective if instituted before organ damage has occurred. Thus, if patients are diagnosed in the pre-cirrhotic and treated, then life expectancy is normal (5–7, 13).

This study represents a model regarding:

a) the identification of the suspected cases using the questionnaire and the website;

b) the confirmation of the diagnosis using the genetic test for the C282Y mutation.

Our website, as an educational and laboratory service, creates the framework for a direct and permanent linkage with physicians and people who are at risk or have been diagnosed with HFE-HH. The eleven tested patients were referred to us by their physicians as a result of the online access. The two heterozygotes displayed mild to moderate clinical and biochemical expression of HFE-HH and the other two patients, who were homozygotes for the C282Y mutation, displayed the full-blown disease (except hepatocellular carcinoma). The genetic test results allowed, in the above cases, the proper guidance of the therapy. Determination of
HFE genotype is clinically useful in patients with suspected iron overload and may lead to identification of otherwise unsuspected C282Y homozygotes.

Finally, we consider that the investigation of iron overload during routine clinical visits, together with the availability of HFE genotyping for family and population studies, will facilitate the early detection of HFE-HH. The knowledge gained from this study will improve the HFE-HH screening methods in order to decrease the morbidity and mortality in this disease.

REFERENCES