Screening for iron overload and HFE mutations in a university hospital

Hereditary hemochromatosis (HH) screening is based on transferrin saturation and serum ferritin concentration. These criteria are used in hospitals although many patients without HH show the same iron abnormalities. Our study detected the HFE mutations in only a few patients (5 out of 55) who had a positive screening test. In hospitals additional criteria (hepatitis, alcoholism, etc.) should be considered.

Sir,

Hereditary hemochromatosis (HH) is probably the most prevalent genetic disorder among Europeans and their descendants.\(^1\)\(^2\) Some studies have shown that the frequency of C282Y mutation ranges from 2 to 3% in our area of Spain.\(^3\)\(^4\) Screening for this condition is based on transferrin saturation and/or serum ferritin measurements.\(^5\) A number of cut-off points have demonstrated good sensitivity and specificity.\(^6\) However, most of these studies have been carried out in primary care centers.\(^3\)\(^5\)\(^6\)

In this regard, patients with a positive screening test were studied in a third level hospital to ascertain whether the HFE-related mutations were implicated.

A prospective blind study was performed on 55 consecutive individuals who were out-patients, aged ≥30 years and showed a transferrin saturation ≥50% and a serum ferritin ≥450 mg/L.

Clinical data concerning iron overload disorders (clinical HH), hematologic disorders and liver disorders were recorded.

The C282Y and H63D mutations of the HFE gene were studied using a polymerase chain reaction.\(^7\) A patient was considered to have HH when homozygosity for the mutation C282Y (phenotype: YY/HH) or compound heterozygosity for the C282Y and the H63D mutations (CY/HD) was demonstrated.

Hepatic disorders were found in 38 cases (69%) (29 hepatitis C virus, 8 alcoholism and 1 with both).

Malignant hematologic disorders were recorded in 13 cases (23.6%), 10 requiring transfusions (6 myelodysplastic syndrome, 3 acute leukemia and 1 idiopathic myelofibrosis). Non-malignant hematologic disorders were demonstrated in 2 cases (3.6%) (1 hereditary xerocytosis and 1 hereditary spherocytosis). Only two cases with HH had been previously diagnosed.

Three patients (5.5%) were homozygous for the C282Y mutation (YY/HH); including the two previously diagnosed cases of HH and 1 non-transfused patient with a diagnosis of refractory ringed sideroblastic anemia (Hb 119 g/L, transferrin saturation 72% serum ferritin 876 µg/L). The patients with hereditary spherocytosis and idiopathic myelofibrosis were compound heterozygous (CY/HD).

The results of the remaining cases are shown in Table 1. One alcoholic and 2 patients with hepatitis C virus infection were homozygous for the mutation H63D (CC/DD).

After the discovery of the HFE gene mutations, some authors demonstrated that the study of HFE mutations is the best way to confirm HH.\(^8\)

Fewer than 10% of our out-patients who had a positive iron overload screening test carried the mutations related to HH. In most cases liver and hematologic disorders accounted for the iron abnormalities.

In the light of our data, other criteria apart from iron overload should be employed to prevent the genetics department of our hospitals from receiving unnecessary patients.

<table>
<thead>
<tr>
<th>Cases (%)</th>
<th>Clinical diagnosis</th>
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<tbody>
<tr>
<td>YY/HH</td>
<td>2 Hereditary hemochromatosis</td>
</tr>
<tr>
<td></td>
<td>1 Ringed sideroblastic refractory anemia</td>
</tr>
<tr>
<td>CY/HD</td>
<td>1 Idiopathic myelofibrosis</td>
</tr>
<tr>
<td></td>
<td>1 Hereditary spherocytosis</td>
</tr>
<tr>
<td>CY/HH</td>
<td>3 Hepatitis C virus</td>
</tr>
<tr>
<td>CD/DY</td>
<td>19 (34.5%) alcohol</td>
</tr>
<tr>
<td></td>
<td>3 Malignant blood diseases</td>
</tr>
<tr>
<td></td>
<td>14 Hepatitis C virus</td>
</tr>
<tr>
<td>CC/DD</td>
<td>3 (5.5%)</td>
</tr>
<tr>
<td></td>
<td>1 Alcoholism</td>
</tr>
<tr>
<td></td>
<td>2 Hepatitis C virus</td>
</tr>
<tr>
<td>CC/DY</td>
<td>25 (45%)</td>
</tr>
<tr>
<td></td>
<td>10 Hepatitis C virus + alcoholism</td>
</tr>
<tr>
<td></td>
<td>5 Alcoholism</td>
</tr>
<tr>
<td></td>
<td>8 Malignant blood diseases</td>
</tr>
<tr>
<td></td>
<td>1 Non-malignant blood diseases</td>
</tr>
</tbody>
</table>

References


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Table 1. HFE mutations results and clinical data from the patients included in this study.
unnecessary samples. However, it should be pointed out that three out of our 16 hematologic cases had the HFE mutations related to HH.

One important question remains to be answered. Is transferrin saturation as good a screening test in Southern Europe as it is in Northern Europe? In this regard, a number of studies have demonstrated a lower prevalence of the C282Y HFE mutation in these areas (from 0.5 to 3%). Moreover, a number of potentially confounding pathologies are more frequent in our countries (hepatitis C virus infection, thalassemia, alcoholism, etc.).

In conclusion, in tertiary level medicine, additional criteria (e.g., hepatitis C virus infection, alcoholism, hematologic disorders, transfusions) together with a positive iron overload screening test are necessary to isolate HH from other pathologies.

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References

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Cytoplasmic overexpression of p53 and p21<sub>ras</sub> in megaloblastic anemia

In this study, we describe, for the first time, cytoplasmic overexpression of p53 and p21<sub>ras</sub> in megaloblastic anemia (MA). Nuclear p53 expression and mutations of p53 and N, H, and K-ras genes were absent. These results suggest that wild-type p53 protein might induce apoptosis in MA and help maintain genetic stability in this disease.

Sir,
We found overexpression of p53 and p21<sub>ras</sub> proteins in a patient with megaloblastic anemia (MA). We studied the expression of p53 and p21<sub>ras</sub> in bone marrow cytosplasts from this patient with MA and in 6 normal control samples from bone marrow donors. The levels of these proteins were assessed by the alkaline phosphatase-anti-alkaline phosphatase procedure (APAAP) with monoclonal antibodies PAb 1801 (p53) and Y13-259 (p21<sub>ras</sub>) (Oncogene Science - Calbiochem). The patient was a 41-year old man with anemia. Laboratory data showed red blood cells 2.54 x10<sup>12</sup>/L, hemoglobin 7.8 g/dL, hematocrit 21.6%, mean corpuscular volume 85 fl, reticulocyte count 1.3%, white blood cells 11.5 x10<sup>9</sup>/L, platelets 414 x10<sup>9</sup>/L, serum folate 3.73 ng/mL (normal 3-17 ng/mL), serum B<sub>12</sub> <100 pg/mL (normal 300-1,000 pg/mL), and serum lactate dehydrogenase 5,954 U/L (normal 150-450 U/L). Bone marrow aspirate revealed typical megaloblastic morphology. A diagnosis of heterozygous α<sub>α</sub> thalassemia was also confirmed by Southern blotting. We found that p53 protein was highly expressed (+++ intensity) in the cytoplasm of all bone marrow cells in the cytosplast (Figure 1), while p21<sub>ras</sub> was moderately overexpressed (++ intensity). No expression was observed in the nucleus. All the control samples exhibited very low levels of p53 and p21<sub>ras</sub> expression. Six months after successful treatment with folate and vitamin B<sub>12</sub>, the patient's p53 and p21<sub>ras</sub> expression was similar to that of controls. Additionally, polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) analysis of the patient's bone marrow sample was negative for mutations in codons 12, 13 and 61 of N-, K-, and H-ras genes as well as in exons 2-11 of the p53 gene.

The patient showed an unusually high expression of wild-type p53 protein (wt-p53) in the cytoplasm of all bone marrow cells at diagnosis as demonstrated by the absence of p53 mutation, while normal bone marrow controls exhibited weak cytoplasmic p53 expression. Nuclear p53 expression was absent. As in our controls, small amounts of p53 were found by Katsumoto et al. in the cytoplasm of normal peripheral blood lymphocytes using the same monoclonal antibody. It has already been shown that exogenous p53 may rapidly and reversibly shuttle between cytoplasm and nucleus, a phenomenon that endogenous p53 can also undergo in response to DNA damaging agents. Thus, cytoplasmic localization does not imply inactivation of wt-p53 functions such as cell cycle arrest or apoptosis. The overexpression of p53 found in this patient may have been triggered by DNA...