The prevalence of hereditary haemochromatosis in a diabetic population

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Summary

Hereditary haemochromatosis is an under-diagnosed and treatable cause of chronic liver disease. Its prevalence indicates that selective population screening may be worthwhile, but opinion differs as to whether diabetic patients constitute such a group. We studied 727 patients attending a teaching hospital diabetic clinic. On first testing, 7.4% had abnormally high iron indices, but only 3% remained abnormal on retesting. Of these patients, those at high risk were offered liver biopsy for histological assessment and iron assay. Only one had hereditary haemochromatosis, but all had abnormal liver histology—largely steatosis but some with fibrosis. These findings raise questions regarding the true prevalence of this disorder in North-East England, do not indicate that targeted screening of diabetic patients is worthwhile, and incidentally highlight the potential importance of diabetes as a cause of liver disease.

Introduction

Hereditary haemochromatosis (HHC) is an autosomal recessive condition whose gene lies on the short arm of chromosome six, near the HLA locus. It causes excessive intestinal absorption of dietary iron, the lifelong accumulation of which results in its deposition in organs including the liver, heart, pancreas and gonads. Untreated patients frequently develop cirrhosis, often complicated by terminal hepatocellular carcinoma. Long-term survival analyses have found a normal survival expectation for patients venaecsted in order to deplete total body iron stores, when the diagnosis was made before the development of cirrhosis. Early detection of asymptomatic homozygous individuals can thus reduce morbidity and improve survival, and screening has therefore been advocated. This is relatively inexpensive and, most importantly, detects a condition whose treatment is safe, simple and effective. The cost-effectiveness of population screening is unknown, and targeting of specific high-prevalence groups may be preferable. As diabetes is prognostically associated with haemochromatosis in 60–80% of patients, people assumed to have idiopathic diabetes may constitute such a group. Phelps et al. in South Australia have suggested that the prevalence of haemochromatosis is significantly increased in diabetic patients but their findings have not been confirmed in European diabetic clinics (O’Brien, 1990, Singh et al., 1992). The aims of the current study was to determine the prevalence of haemochromatosis amongst unselected diabetic patients in the relatively homogenous and stable population in North East England, and to evaluate the efficacy of screening such patients routinely.

Since the completion of this study the cloning of a candidate gene, HLA-H, two mutations of which were found in 87% of patients with HHC, has been reported, and a simple PCR-based test to detect homozygosity in DNA from > 80% of individuals is now feasible.

Methods

Patients

Over a 6-month period, all non-pregnant diabetics aged over 16 years, and attending the Diabetes
Clinic at the Royal Victoria Infirmary, were offered screening by sampling 15 ml venous blood under non-fasting conditions. Informed verbal consent was obtained, and the study was approved by the local joint ethics committee.

**Screening**

Serum ferritin was measured by a double-antibody technique on a Stratus immunoanalyser using a commercial kit (Baxter Diagnostics). Serum iron was measured on an Olympus 560 analyser set at 590 nm, using 2,4,6-tripyridyl-S-triazine reagent. Male patients with a serum ferritin >350 μg/l, females with a serum ferritin >250 μg/l and any patient with a transferrin saturation >55% were recalled for repeat sampling. If their results remained indicative of iron overload, then liver biopsy was offered after full clinical assessment. Written, informed consent was obtained, and biopsies were performed under ultrasound guidance, using a modified Tru-cut needle and ‘Biopty’ gun. In patients aged >70 years, in whom the initial tests indicated only equivocal iron overload, HLA testing was done in lieu of liver biopsy, which was not deemed ethically justifiable. The liver biopsy sample was divided for histology and liver iron estimation. Sections were routinely cut at 4 μm from formalin-fixed, paraffin-embedded samples and stained with haematoxylin and eosin, Perls, Van Giesen, Foots reticulin, PAS (with and without diastase) and Shikata. The Perls stain was assessed in a semi-quantitative method as described by Scheuer. Liver iron content was measured by flame atomic absorption spectrophotometry at a wavelength of 248.3 nm. Liver (2–8 mg wet weight) was dried at 100°C to a constant weight, dissolved in concentrated nitric acid and hydrogen peroxide, and digested in a 700 W microwave oven before dilution and analysis. Recovery from standards was 96–102%, and a reference range was established by analysis of post-mortem liver specimens with no histological evidence of haemochromatosis. Measurement of liver iron levels allowed calculation of the liver iron index (hepatic iron concentration in μmol/g dry weight/age). Values >1.9 were highly suggestive of homozygous disease; values <1.5 were regarded as normal.

**Results**

We screened 727 patients (405 male) aged 19–91 years (mean 58.3 years); 413 had type 1 diabetes and 314 type 2. Over 95% were Caucasian. The results of the screening strategy are summarized in Table 1, and the characteristics of patients undergoing liver biopsy, in Table 2.

Initial screening yielded 54 patients (7.4%) with possible iron overload, 39 of whom had an elevated serum ferritin, 13 a transferrin saturation >55%, and two with both criteria abnormal. Of the 39 with an elevated serum ferritin (21 male, mean age 61.5 years) 20 had normal iron indices on repeat sampling. Four females aged >60 years had a repeat ferritin between 250 and 350 μg/l. All four were HLA typed, and none carried types A3 or B7 (associated with 70% of cases of genetic haemochromatosis). They were not offered biopsy. Five males aged >65 years had a repeat ferritin lying between 350 and 500 μg/l. All had normal tests of liver function, were deemed to be of low risk and thus none underwent liver biopsy. The remaining ten patients, all of whom had type 2 diabetes, had persistently raised ferritin values and underwent liver biopsy. Sufficient tissue to allow liver iron estimation was obtained in seven, in all of whom the liver iron index lay below 1.5. Histologically, four of the ten had Grade 1 haemosiderosis of no significance and none had haemochromatosis. However, all ten had macrovesicular steatosis, and six had at least early fibrosis.

Of the thirteen patients with an elevated transferrin saturation alone (all male, mean age 40.2 years) eleven had normal values on repeat, one declined re-screening (26-year-old male, transferrin saturation 68%, serum ferritin 63 μg/l) and one underwent liver biopsy. This was a 22-year-old, insulin-dependent diabetic male, in whom biopsy confirmed the presence of pre-cirrhotic haemochromatosis.

Two patients had both abnormal ferritin and transferrin saturation on initial screening. On recall, one of these, a 63-year-old male with insulin-dependent diabetes and a history of alcohol excess, had an elevated ferritin only and transferrin of 55%. Biopsy showed grade 3 haemosiderosis, macrovesicular steatosis and mild fibrosis but a normal liver iron index of <1. The other, a 72-year-old male with type 2 diabetes, had an elevated ferritin only on repeat (450 μg/l) and declined biopsy.

Forty-seven patients were iron-deficient, and were investigated separately. In summary, of 727 patients, 22 had abnormal iron indices on repeat screening, of whom only one had proven hereditary haemochromatosis by histological and biochemical criteria.

In light of the steatosis noted on liver biopsy, further data were collected on ten of the patients biopsied. The values given are means (SD). They were of mean age 59.6 years (10.6), weight 90.6 kg (15), body mass index 31.1 kg/m² (3.6). This differs significantly from the mean weight of a consecutive series of 1086 patients with NIDDM attending the same clinic (75.4 kg (15.7), p < 0.01; BMI 28.2
Table 1  Screening strategy in 727 patients

<table>
<thead>
<tr>
<th>First screening</th>
<th>n</th>
<th>Second screening</th>
<th>Biopsied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>626</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Iron-deficient</td>
<td>47</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Elevated ferritin</td>
<td>39</td>
<td>Normal 20, low risk 9, elevated ferritin 10</td>
<td>10</td>
</tr>
<tr>
<td>Elevated transferrin saturation</td>
<td>13</td>
<td>Normal 11, declined rescreen 1, elevated transferrin 1</td>
<td>1</td>
</tr>
<tr>
<td>Elevated ferritin &amp; transferrin saturation</td>
<td>2</td>
<td>Elevated ferritin only 2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2  Characteristics of patients undergoing liver biopsy

<table>
<thead>
<tr>
<th>Abnormality on recall</th>
<th>Age, sex</th>
<th>Ferritin (µg/l)</th>
<th>Transferrin saturation (%)</th>
<th>Alcohol consumption (units/week)</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated serum ferritin</td>
<td>68, M</td>
<td>518</td>
<td>25</td>
<td>15</td>
<td>Steatosis, grade 1 haemosiderosis</td>
</tr>
<tr>
<td></td>
<td>65, M</td>
<td>396</td>
<td>28</td>
<td>50+</td>
<td>Steatosis, fibrous septae</td>
</tr>
<tr>
<td></td>
<td>64, M</td>
<td>409</td>
<td>16</td>
<td>20</td>
<td>Steatosis</td>
</tr>
<tr>
<td></td>
<td>58, M</td>
<td>564</td>
<td>53</td>
<td>10</td>
<td>Steatohepatitis, early fibrosis, grade 1 haemosiderosis</td>
</tr>
<tr>
<td></td>
<td>57, F</td>
<td>382</td>
<td>27</td>
<td>50+</td>
<td>Steatosis, fibrosis, mild chronichepatitis</td>
</tr>
<tr>
<td></td>
<td>57, F</td>
<td>364</td>
<td>30</td>
<td>&lt;10</td>
<td>Steatosis, mild fibrosis</td>
</tr>
<tr>
<td></td>
<td>52, M</td>
<td>384</td>
<td>39</td>
<td>15</td>
<td>Steatosis</td>
</tr>
<tr>
<td></td>
<td>73, M</td>
<td>1200</td>
<td>55</td>
<td>12</td>
<td>Steatosis, mild fibrosis, grade 1 haemosiderosis</td>
</tr>
<tr>
<td></td>
<td>60, M</td>
<td>516</td>
<td>45</td>
<td>20+</td>
<td>Steatohepatitis, mild fibrosis</td>
</tr>
<tr>
<td></td>
<td>42, M</td>
<td>1390</td>
<td>40</td>
<td>Nil</td>
<td>Steatosis, grade 1 haemosiderosis</td>
</tr>
<tr>
<td></td>
<td>63, M</td>
<td>396</td>
<td>55</td>
<td>50</td>
<td>Steatosis, mild fibrosis, grade 3 haemosiderosis</td>
</tr>
<tr>
<td>Elevated transferrin saturation</td>
<td>22, M</td>
<td>323</td>
<td>79</td>
<td>16</td>
<td>Haemochromatosis</td>
</tr>
</tbody>
</table>

(4.8), \( p=\text{NS} \)). The biopsied patients had a mean duration of diabetes of 13.3 years (6.3), a haemoglobin A1c value of 6.9% (1.3), plasma cholesterol 6.0 mmol/l (1.4) and triglyceride 3.3 mmol/l (1.7). Two patients were on insulin.

Discussion

The population prevalence of genetic haemochromatosis in the UK is uncertain but geographically disparate studies\(^{14,15}\) yield figures of 3–8 per 1000, implying a gene prevalence of about 10%.\(^{16,17}\) It is one of the commonest inherited metabolic disorders amongst the White population worldwide but is probably underdiagnosed due to its non-specific presenting features.

A major difficulty with screening programmes to date has been determining the most appropriate test of iron overload.\(^{18}\) In non-fasted patients, a threshold of 62% for transferrin saturation is unsatisfactory as a single test, as up to 83% of subjects later test normal.\(^{19}\) Similarly, serum ferritin alone is a poor marker as it rises with age or with acute illness, is normal in some probands subsequently found to be homozygous,\(^{19}\) and may be elevated in diabetes uncomplicated by haemochromatosis.\(^{20}\) In a study of 120 young first-degree relatives of haemochromatosis probands, (Bassett et al.\(^{21}\)) a combination of serum ferritin plus transferrin saturation was the most reliable screen, with a sensitivity of 94% and specificity of 86%. Beilby et al.\(^{22}\) however have suggested that the calculated transferrin index (serum iron/transferrin) should replace transferrin saturation in screening studies. To maximize the chances of detecting homozygous carriers, and to permit direct comparison with previous studies, we chose the Bassett criteria for this study. The study reported here
is the largest such survey, and examines a relatively genetically homogenous population. Of the 727 diabetic patients screened 22 (3%) had persistently abnormal iron indices, but only one of these had hereditary haemochromatosis by histological or biochemical criteria. Assuming a population prevalence of 4/1000, we would have anticipated detecting approximately three cases in a group this size. Whilst this may simply be related to chance or the previous selecting-out of known cases, this low prevalence raises questions as to the true prevalence of the condition in North-East England. Haplotype analyses used to refine the position of the HHC gene suggest a strong founder effect exerted by a common Celtic ancestor, and thus different White populations worldwide may have variable population frequencies, depending on their ancestry. Approximately a quarter of patients with haemochromatosis have diabetes at the time of presentation, although it is a presenting feature in less than 2%. Type 1 diabetes and haemochromatosis do not share common HLA associations, but there is clearly a complex link between iron overload and diabetes. The risk of diabetes is increased in these patients if there is a family history of type 2 diabetes, presumably being a summation of two pathological processes in the pancreas. Despite this close association between the two conditions, we have failed to demonstrate any increased prevalence of iron overload amongst the population examined. The screening criteria adopted are at least as sensitive as those used in comparable studies, and yielded 22 patients with persistently abnormal iron indices, only one of whom had homozygous disease. This confirms the low positive predictive value of an elevated ferritin or transferrin saturation in diabetics, and accords with the findings of smaller studies from London and Cork. Even selecting only those diabetics with abnormal liver function yields only the predicted rate of haemochromatosis for the general population. In contrast, Phelps et al. reported an apparently increased prevalence amongst their south Australian diabetic population of 418 patients, whose mean age and sex compare to those reported here, but with a lower proportion of patients with type 1 diabetes (30% vs. 57%). The discrepancy between the Australian and European studies may reflect geographic variation in disease prevalence, with a greater influence of the founder effect in the Australian population, although the findings of Phelps et al. could simply be a chance finding, as their total number of haemochromatosis patients was only four.

One unexpected finding in the present study was the high proportion of patients exhibiting fibrosis in their liver biopsy. Hepatic dysfunction, especially steatosis, has long been recognized in diabetes, and may progress, as the prevalence of cirrhosis in diabetics at autopsy is twice the population average. Although our patients had relatively well-controlled serum lipids, their mean body mass index was just into the range defined as obese, and these patients, selected for biopsy on the basis of their iron status, were significantly heavier than the rest of the non-insulin-dependent population. Steatosis is not characteristically associated with abnormal iron indices, but obesity and its related disorders, especially diabetes, may cause non-alcoholic steatohepatitis. This poorly-defined condition is characterised by steatosis, an inflammatory infiltrate and early fibrosis. It may progress to cirrhosis in a small minority of patients, but the severity of liver damage does not appear to correlate directly with obesity, hyperlipidaemia or hyperglycaemia. It is therefore possible that iron may have contributed to the liver abnormalities in the study patients, as iron may cause hepatocyte damage by lipid peroxidation and increased lysosomal fragility, and act as a direct stimulus to collagen synthesis. Obesity in diabetes as a risk factor for hepatic fibrosis warrants further study.

Targeted screening for haemochromatosis in diabetic clinics does not appear to be a more effective exercise than whole population screening as the condition accounts for less than 1% of patients with diabetes, and around 0.3% in the present study. Whilst haemochromatosis should be considered in the differential diagnosis of diabetics with abnormal liver function tests, such patients are more likely to have steatosis and possibly fibrosis directly as a result of their diabetes. The important issue of early detection of HHC will soon be addressed by the ‘new age’ of applied genetics.

Acknowledgements

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References

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